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A COMPARISON OF SOME ENZYMES CATALYSING BIOTIN-DEPENDENT REACTIONS

By M. H. BRIGGS, Department of Chemistry, Victoria University of Wellington, New Zealand

(Received for publication, 30 November 1959)

Summary

Highly active preparations of the three enzymes oxalacetic carboxylase, hexokinase, and the ornithine-citrulline enzyme, all of which catalyse reactions known to be biotin-dependent, were assayed for bound-biotin and activity in the presence of biotin analogues. All three enzymes contained small amounts of bound-biotin but were not inhibited by the biotin analogues, nor stimulated by biotin. It is concluded that if biotin is part of a bound coenzyme for these enzymes, it must be in a microbiologically inactive form.

INTRODUCTION

The relationship of biotin to enzymes is largely unknown. A large number of metabolic reactions are absent or reduced in biotin-deficient cells while other reactions may be inhibited by biotin analogues. Some of this work has been reviewed by Lichstein (1951) and by Gyorgy (1954). However, the mechanisms by which biotin influences these reactions are obscure, and the experimental evidence is contradictory. Lichstein (1949a and b) has produced evidence for a coenzyme function for biotin in some systems, but Sund *et al.* (1958) have published findings which support the view that biotin is concerned in enzyme synthesis.

Several attempts have been made to decide between the two alternative hypotheses, the most obvious method being to determine the effects of added biotin to purified preparations of the various enzymes. Ochoa *et al.* (1947), for example, were unable to stimulate the activity of the "malic enzyme" from biotin-deficient tissues, while Chambers and Delwiche (1954) have reported similar findings for a bacterial succinic decarboxylase.

The results are compatible with the view that biotin participates in the synthesis of the enzymes concerned, or that it is part of a larger coenzyme molecule which is required by the apoenzyme.

On the coenzyme hypothesis it would be expected that the coenzyme is either bound to the apoenzyme, in a manner analogous to the flavo-proteins, or is merely required in solution by the apoenzyme. Thus a pure preparation of a biotin-dependent enzyme should either contain a bound-biotin coenzyme, or be inactive in the absence of a solution containing the coenzyme. This latter alternative can probably be dismissed, as many active, pure preparations of biotin-dependent enzymes have been reported. The coenzyme hypothesis, therefore, appears to predict the occurrence of a bound-biotin compound in these enzymes. On the alternative enzyme-synthesis hypothesis no such compound should be present.

Several attempts have been made to detect the possible presence of bound biotin in biotin-dependent enzymes. The results, however, present a confusing picture. Ochoa *et al.* (1947) have reported that highly active preparations of the "malic enzyme" contain only traces of biotin, while Ravel *et al.* (1959) found only 0·19 millimicro grams (m μ g) of biotin per milligram (mg) of protein for the ornithine-citrulline enzyme of *Streptococcus lactis*. Several workers have reported failure to detect biotin in oxalacetic carboxylase preparations, but Lichstein (1955 and 1957) has found an apparently significant correlation between the degree of purity of this enzyme and its content of bound-biotin. He claims that the failure of previous workers to detect this relationship may be due to their insensitive assay techniques. The largest amount of bound-biotin detected by Lichstein was approximately 3·0 m μ g per mg of enzyme protein. In other studies the present authors found similar amounts of bound-biotin in a crystalline hexokinase preparation, but the significance of this value is questionable as other biotin-independent enzymes contained similar quantities.

The largest amount of bound-biotin so far reported is 200 to 250 m μ g per mg protein for an enzyme fraction of avian liver which participates in fatty acid biosynthesis (Wakil *et al.*, 1958).

The present study was undertaken to obtain pure preparations of the three biotin-dependent enzymes, oxalacetic carboxylase, hexokinase, and the ornithine-citrulline enzyme. The bound-biotin contents of these preparations were determined and attempts to inhibit their activity with biotin analogues were made.

METHODS AND MATERIALS

All microorganisms used in this study were obtained from the American Type Culture Collection, Washington, D.C. Of the biotin analogues used, norbiotin and homobiotin were the gift of Hoffman-La Roche, Inc., Nutley, N.J., U.S.A., dethiobiotin was purchased from the Nutritional Biochemicals Corporation, and biocytin was the gift of Dr L. D. Wright of Cornell University, Ithaca, N.Y., U.S.A.

Oxalacetic carboxylase was prepared by a method based on the procedure of Utter and Kurahashi (1954). Fresh chicken livers were homogenised with acetone at 5°C and then dried overnight at the same temp. over concentrated sulphuric acid. After removal of connective tissue, the resulting

powder was triturated with 0·001 M phosphate buffer at pH 7·8 and incubated at 38°C for 15 minutes. From here the procedure of fractionation with ammonium sulphate and then treatment with calcium phosphate and alumina gels followed the original method. The activity of the preparation was assayed by determining the rate of $C^{14}O_2$ incorporation into a pool of oxalacetic acid (OAA) in the presence of adenosine triphosphate (ATP) and manganese ions by the method of Utter (1951). Specific activity is expressed in terms of micromoles $\times 10^{-2}$ of CO_2 fixed per minute per milligram of protein. The oxalacetic carboxylase prepared for this study had a specific activity of 32·8.

The ornithine-citrulline enzyme was prepared by the method of Ravel *et al.* (1959). Ten litres of a 24 hour culture of *Streptococcus lactis* 8039 were harvested by centrifuge, washed in Tris buffer, pH 8·1, and converted in portions to a cell-free extract by a sonic oscillator. Cell debris was removed by centrifugation. The solution was treated with saturated ammonium sulphate at room temperature for 30 minutes. The precipitate was removed by centrifugation and discarded. The supernatant was cooled to 4°C and a calculated quantity of ammonium sulphate added to produce a 75% saturated solution. The precipitate was collected and suspended in 0·05 M phosphate buffer, pH 7·5. The solution was dialysed against the same buffer for several hours and then heated to 65°C by a water bath for 5 minutes. The denatured protein was removed. After a further similar fractionation with ammonium sulphate a precipitate was obtained which was taken up in 0·015 M glycine buffer, pH 8·5. This solution was dialysed against the same buffer for several hours at 4°C. Aliquots of this solution were then added to columns of DEAE-cellulose, type 20. After washings with various concentrations of glycine and phosphate buffers, pH 8·5, the adsorbed enzyme was removed by a buffer mixture at pH 7·5. Enzyme activity was determined by the method described by the previous workers (Ravel *et al.*, 1959), and is expressed in terms of micromoles of citrulline produced per milligram of protein per hour. The specific activity of the enzyme used in the present study was 71,000.

Hexokinase was recrystallised by the methods of Kunitz and McDonald (1946) from a sample of yeast hexokinase provided by the Sigma Chemical Company. The activity was 1400 KM units per mg protein.

Biotin assays were conducted with *Saccharomyces cerevisiae,* strain 139, and *Lactobacillus arabinosus* 17–5, using the techniques of Hertz (1943) and Wright and Skeggs (1944) respectively. Bound-biotin was released by hydrolysis with 3·6 N sulphuric acid at 121°C for 1 hour.

Enzyme activity of all preparations was determined alone, and in the presence of 10 micrograms per mg of protein of each of the following, *d*-biotin, *d*-dethiobiotin, *d*-norbiotin, *d*-homobiotin and *d*-biocytin.

RESULTS

TABLE 1—Bound-biotin Contents of Enzymes

Enzyme	Bound-biotin m μ g/mg protein
oxalacetic carboxylase	2·91
hexokinase	2·75
ornithine-citrulline enzyme	0·24

The bound-biotin contents of the three enzymes are given in Table 1. The results are derived from the yeast assay which gave slightly higher values than the *L. arabinosus* assay. The biotin contents of the oxalacetic carboxylase and the ornithine-citrulline enzyme are similar to the previously reported values.

TABLE 2—Properties of Enzymes

Enzyme	Optimum pH	Inhibited by
oxalacetic carboxylase	5·9–6·3	p-chloromercuribenzoate
hexokinase	8–9	.....
ornithine-citrulline enzyme	8·3–8·9	orthophosphate

Other properties of the enzymes derived during these experiments are listed in Table 2.

It was observed that, within the experimental error, no alteration in activity of any enzyme occurred on treatment with biotin or biotin analogue. Concentrations of up to 10 micrograms per mg of enzyme protein were tested, with and without preincubation for one hour.

CONCLUSIONS

It is apparent that none of the three enzymes used in this study is affected by biotin or it analogues in solution at the concentrations tested. Hence if a biotin-coenzyme is required it must be tightly bound to the enzyme so that analogues cannot compete for the active site. Yet on acid hydrolysis very little biotin can be detected. It therefore follows that if a biotin-coenzyme exists it cannot be released from the enzyme by acid hydrolysis in a microbiologically active form.

The present experiments demonstrate either some properties of the hypothetical coenzyme, or can be regarded as evidence against the coenzyme hypothesis. Experiments are now being conducted to release the bound-biotin from the enzyme by alternative methods to examine further the coenzyme hypothesis.

A final possibility remains. It is clear from the observed concentrations of bound detectable biotin in these enzymes that many of the enzyme-protein molecules cannot be associated with a normal biotin molecule. However it is possible that only those which are have any enzymatic activity. This hypothesis could be tested by separating the biotin-containing molecules from the remainder and determining the activity of the two fractions. Unfortunately this experiment would be most difficult to perform.

The mechanism of the action of biotin remains unknown, but the evidence presented above renders the coenzyme hypothesis unlikely, though not impossible.

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NOTES ON A PRELIMINARY INVESTIGATION INTO THE HABITS AND LIFE CYCLE OF *COPRIS INCERTUS* SAY (COPRINI: COLEOPTERA) IN NEW ZEALAND

By W. P. THOMAS, Entomology Division, Department of Scientific and Industrial Research, Nelson

(Received for publication, 28 September 1959)

Summary

The habits and life cycle of the introduced beetle, *Copris incertus* Say were studied to ascertain its possibilities as an agent in the control of noxious species of flies, its probable rate of development, and chances of establishment and spread in New Zealand.

This species, which is of Mexican origin, was introduced into New Zealand from Western Samoa where it plays a significant part in the reduction of flies by removal, in part, of the animal droppings in which flies breed. In New Zealand, the fly most likely to be reduced in this manner is *Sarcophaga milleri* Johnston & Hardy, which is probably not of primary importance to either stock or to human health. The relative importance and relevant habits of the more important flies are discussed.

Duration of life cycle under controlled and insectary conditions indicates that in New Zealand this insect is not likely to multiply fast enough to have any significant effect on fly populations. Development from egg to adult at 75°F occupied approximately 62 days.

INTRODUCTION

Adults of *Copris incertus* (Fig. 4) were first introduced into New Zealand in November 1955 and in February 1956 (through courtesy of Dr R. A. Cumber) and October 1957 and January 1958 (through courtesy of Mr P. Dale). This material was all imported from Western Samoa. The Coprini ("dung beetles") by virtue of their burying of animal faeces for nourishment of their larvae tend to reduce a potential fly breeding medium. It is also possible that this dung burying activity is beneficial in soil aeration and in the placement of a valuable pasture nutriment in a useful position.

Sarcophaga milleri, the principal fly breeding in cattle droppings in New Zealand, is of secondary importance as a nuisance to stock. *Muscina stabulans* (Fall.) also breeds to some extent in stock droppings and is also of secondary importance. The most important fly as regards influence on stock (particularly dairy cattle) in New Zealand is *Stomoxys calcitrans* (L.). It is well known that this fly is not attracted to unaltered cattle droppings, but breeds extensively in decaying vegetable matter, especially where it is mixed with horse manure or fluid slush from other faecal matter. It is thus not likely to be influenced by *Copris.* The histerid beetle *Pachylister chinensis* (Quens.) which is predaceous on dipterous larvae is more likely to be effective against this species (Simmonds, 1958).

Copris incertus has become well established on some of the tropic Pacific Islands, and in these areas it plays a part in the reduction of fly populations (Simmonds 1958).

In New Zealand, field releases have been made in the Whangarei and Nelson districts but no evidence of establishment has been noted. However, in December 1958 a further 19 males and 24 females were caged over cattle droppings on a Nelson farm and at the time of writing (July 1959) tunnelling activity indicates that these insects can survive under both New Zealand summer and winter conditions. Fresh cattle droppings are supplied regularly to this nucleus colony.

HABITS OF ADULTS

Observations on habits and life cycle of *Copris incertus* are made difficult by the nocturnal nature of most above-ground activity and subterranean diurnal activity.

Almost immediately after release of adults on to a suitable substrate, during the diurnal period the condition of feigned death is terminated and burrowing ensues, the insect using the extended clypeus with its bordering ocular lobes or genae and toothed fore-tibiae for leverage. Under seemingly natural conditions in an insectary where releases were made adjacent to fresh cattle droppings it was noted that although some beetles tunnelled directly through the droppings, the majority tunnelled at the perimeter of the dung to reach the soil. A few moved several inches away from the droppings before tunnelling.

The presence of tunnels is usually characterised (especially in well consolidated soils) by conical mounds of soil adjacent to droppings.

Tunnels vary considerably in length from a few to several inches and their diameter varies according to the size of the beetle. All tunnels examined have been oblique and have been found to terminate in a large more or less horizontal cavernous chamber.

The gathering and moving of dung from the ground surface has not been observed for this species, but J. H. Fabre (1897) gives a very full account of this process for other members of the Coprinae. In the case of *Copris incertus* the present writer found that the first inch or more of the tunnel is commonly lined with dung. On exposing the terminal cavernous portion of six tunnels after three nights following exposure of dung to beetles, it was found that in each case a mass of dung many times larger than the beetles occupied the greater part of each cavity. The size and shape of the dung mass varied considerably from tunnel to tunnel. Four tunnels each contained a female beneath the edge of the dung mass, while in the other two tunnels both male and female beetles were present. Although there was a variation in size and shape of dung masses, in all cases the surfaces were smooth and rounded, dorsally and dorso-ventrally, with an air space between the dung mass and the upper and lateral surfaces of the terminal chamber. (See Fabre 1897, Chap. VII for a comparison with *Copris hispanus* L.)

The above observations were made during October and early November when early seasonal activity was at its height. A second period of superficial activity occurred during the autumn when the next generation of beetles emerged. This autumn activity decreased greatly as temperatures

dropped and during the winter very little activity was apparent, only one or two fresh tunnels being seen. Investigations by Lindquist (1933) of the fall (autumn) tunnels of *Copris tullius* Oliv. showed the tunnels to end blindly and to contain no masses of stored dung. The same seems to apply in most cases to *Copris incertus* although a number of autumn tunnels have been found to contain large masses of dung in the terminal chamber. Lindquist also found that there was an increase in the amount of dung gathered when both sexes were present in the same tunnel and this also seems to be usually the case with *C. incertus*.

Dung deposited in terminal chambers during the early seasonal period of activity remained for several weeks intact except for very minor quantities which were apparently eaten by the adults. This lull in activity may represent a period during which the dung matures to a suitable condition for further use (Fabre 1897). This period of inactivity was not present when reasonably fresh dung was used during a laboratory life cycle experiment.

During December (presumably after mating, which has not been observed) the large stored masses of dung are divided up into smaller spherical or subspherical balls in which eggs are deposited. Only females have been observed engaged in this subdivision of dung masses. The egg is inserted within a cavity specially prepared on a projecting surface of the large dung mass. Dung surrounding the cavity is then moulded over to form a chamber, the upper crust of which is thin and fibrous, to allow aeration of the egg. The ball is afterwards built around the egg chamber by separation from the large mass of dung (Fig. 1a, b). From two to seven balls have been noted

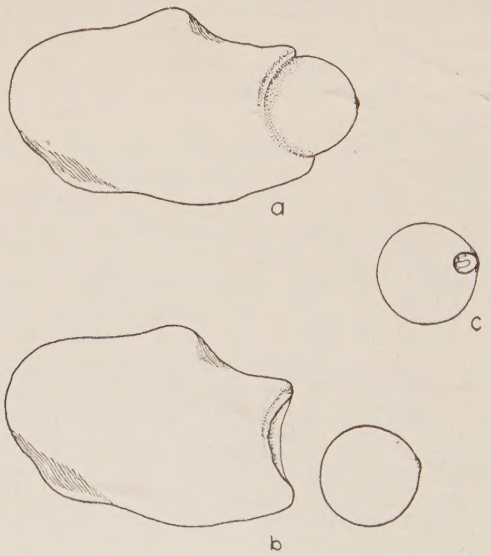

FIG. 1—Stages in the subdivision of a mass of stored dung.
(a) A single dung ball with its contained egg chamber and egg just prior to its separation from the dung mass.
(b) The dung ball after separation.
(c) A median section of a dung ball through the oviposition chamber.

in single tunnels and in one case only has a ball been found to contain two eggs. In this case only one larva developed. The size of dung balls is variable but is usually in the vicinity of 1·8 to 2·5 cm in diameter. While the shape is almost spherical, this depends to some extent on the space available in the terminal cavity, balls sometimes being rather flattened.

The egg, which is elongate and somewhat curved, is approximately 4·2 mm long and 1·7 mm in diameter several hours, (up to twelve) after laying. According to Arrow (1931) the eggs of members of the Coprini increase in size by the imbibation of water before hatching. Each egg is attached by a secretion to the inner surface of the cavity, with it longitudinal axis disposed radially in relation to the dung ball (Fig. 1c). The egg chamber which is eccentric to the ball, is usually indicated externally by a slightly raised area. After oviposition and construction of dung balls the female remains in the terminal chamber during larval development.

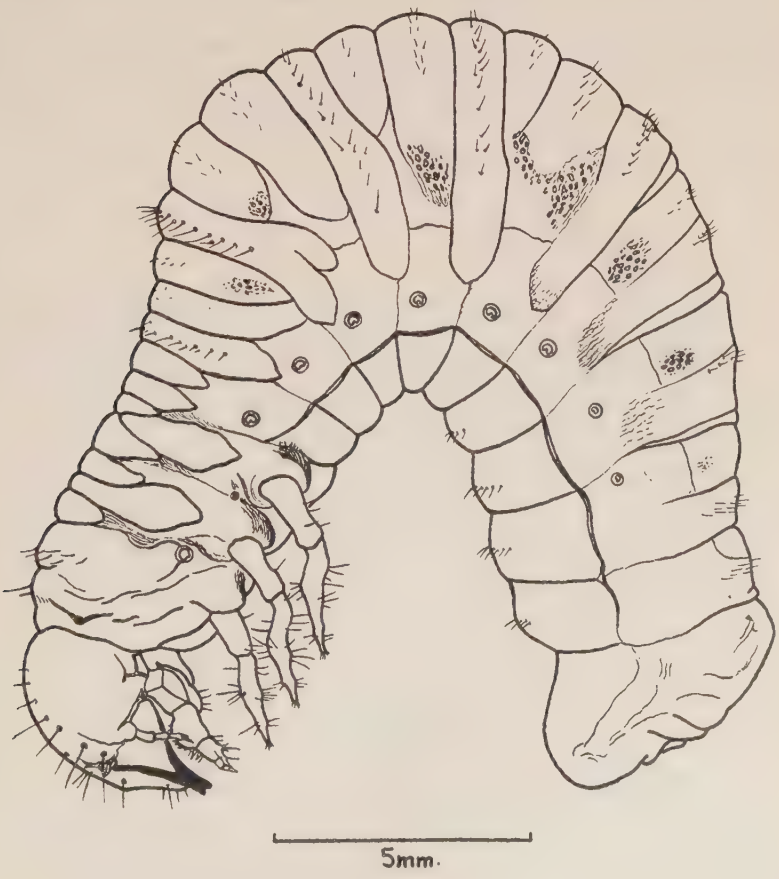

FIG. 2—Final (third) instar larva of *Copris incertus*.

LIFE HISTORY

The total period of development varies considerably in different situations and appears to be largely dependent on temperature and humidity. In Nelson under insectary conditions closely approximating those of the field, the period of development of the summer generation is about 3½ months.

A trial using fresh dung balls containing eggs was carried out in an incubator at a temperature of 75 ± 2°F and high humidity. On 3 December 1958, in order to obtain eggs, a number of females were selected and placed in separate containers of dampened sand and cattle droppings. The sand proved unsatisfactory as a substrate and a week later was replaced by turf. By lifting the turf each container was checked daily (except weekends) for dung balls. On the morning of 15 December 1958 the first balls appeared. Each ball was placed in a muslin-covered glass jar 2½ by 1 inch in size, which was in turn dated and placed in the incubator.

Of a total of 26 eggs laid during a period of ten days, 13 failed to develop beyond the first larval instar. The majority of these did not hatch and the eggs were found to be collapsed. Ten larvae reached the final (third) instar (Fig. 2) and pupated (Fig. 3). Of these, two succumbed to fungal infection and eight finally emerged as adults.

The duration of the cycle from oviposition to adult emergence varied from 57 to 70 days with a mean of 62 days.

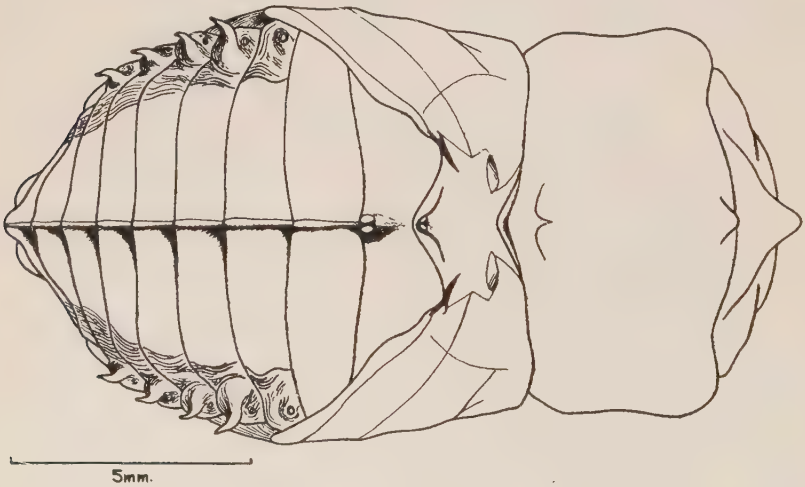

FIG. 3—Pupa of *Copris incertus* viewed from the dorsal surface.

HABITS AND CHARACTERISTICS OF DEVELOPING LARVAE

The entire larval and pupal life is spent within the cavity of the dung ball. As the first instar larva commences to feed the oviposition chamber is enlarged in accordance with food intake and increase in larval size. The feeding larva is bent in the form of a U. The thorax, during this period,

although of not much greater diameter than the head capsule is clearly differentiated from the abdomen by the increase in diameter of the first two abdominal segments and the marked distension of the third and following segments which house the voluminous intestine. Gut contents are normally retained until the larva reaches maturity, but if a puncture occurs in the dung ball, this is quickly repaired by the exudation of larval faecal matter. The larval legs are poorly developed and of little apparent value.

During the early stages of development, the larva is whitish with the gut contents showing through the abdominal walls and fat body as dark patches. The head capsule is light brown, the mandibles much darker. As feeding approaches completion the fat body increases, obliterating the gut contents. On completion of feeding the larva becomes a uniform cream colour except for the darker head, the body shrinks considerably and its U-shape becomes much less pronounced coincident with the exudation of remaining faecal matter, which is used for lining the pupal cell. Shortly before pupation the body develops a transparent layer beneath the segmental folds. At this stage the final larval skin is shed exposing the pale cream coloured pupa which darkens to a pale brown as the integument hardens. After a period of several days the pupa, especially the head and thorax, darkens considerably and features characteristic of the adult beetle can be clearly seen.

The newly emerged adult beetle is very soft, the red-brown head, thorax, and legs being harder than the distended abdomen and much shrunken folded elytra, which are cream in colour. It is the relatively hard legs which free the anterior portion of the body from the pupal skin, the front legs being used as anchors as soon as freed, the remaining legs thrusting the skin

FIG. 4—Adults of *Copris incertus* showing the variation in size of beetles. Top row: Males. Bottom row: Females.

back from the head and thorax. The newly emerged adult remains quiescent within the pupal cell of the dung ball for several days until the chitin has completed hardening and colouring (Fig. 4).

The length of adult life is not known but is probably several months, the actual period depending on climatic conditions.

DISCUSSION

Due to the slow rate of development under New Zealand conditions, *Copris incertus* may take several years to reach appreciable numbers, if it should establish at all. However, evidence indicates survival to be possible here, and ultimate establishment and distribution may be achieved.

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A STUDY OF THE PHYSIOLOGY OF *SPORIDESMIUM BAKERI* SYD.—1. NUTRITION

By D. J. Ross, Soil Bureau, Department of Scientific and Industrial Research, Wellington

(Received for publication, 1 September 1959)

Summary

The optimum temperature for growth of the fungus *Sporidesmium bakeri* Syd.,* which causes facial eczema disease in sheep, was about 24°C and for spore production about 28°C. The fungus could grow over a wide pH range.

The fungus had simple nutritional requirements. Its growth in a synthetic medium was stimulated by the addition of trace metals. Growth factors sometimes increased the rate of growth but did not affect maximum yields in synthetic media. The fungus could utilise pectin, hemicellulose, cellulose, simple carbohydrates and some aromatic acids but grew poorly with aliphatic acids as substrates. Nitrogen present in ammonium, nitrate, or organic compounds was readily utilised.

Spores were produced more abundantly on non-defined than on defined media. γ-Amino-n-butyric acid and asparagine favoured spore production by a high-sporing strain in a synthetic medium.

Introduction

Recent work has established that cultures of *Sporidesmium bakeri* Syd., isolated from pastures in the North Island of New Zealand, cause symptoms of facial eczema disease on feeding to sheep and laboratory animals (Thornton and Percival 1959; Percival 1959; Thornton and Ross 1959). *Sporidesmium bakeri* has been recently recorded also by Janes (1959) as one of the fungi associated with pastures in southern Victoria, Australia. The majority of strains of this fungus have, however, so far been isolated from plants in tropical countries (Hughes 1953).

Some of the nutritional properties of strains of *S. bakeri* isolated from pasture plants and from spore-bearing material in toxic pastures in New Zealand are determined in the present investigation. Thornton and Ross (1959) have already established that *S. bakeri* can grow on defined media but that growth was more vigorous on non-defined media.

The effect of cultural conditions on sporulation is also noted in this study. The demonstration of the production of "beaker test" compound (Percival and Thornton 1958) and of toxin (Thornton and Percival 1959; Percival 1959) by the high-sporing strain C may indicate that the production of these compounds is associated with sporulation. In the field, spores have been observed in high numbers in toxic pastures (Thornton and Ross 1959; Thornton and Sinclair 1959) and it is probable that sheep grazing in the field ingest the fungus mainly in the spore form.

*Recently, Hughes (1958) has proposed the new genus *Scheleobrachea* and has indicated that *Sporidesmium bakeri* Syd. is a facultative synonym for *Scheleobrachea echinulata* (Speg.) Hughes.

It is hoped that results of the present study will provide a basis for the subsequent investigation of toxin production by the fungus in defined media and will also contribute to an understanding of some of the factors affecting the distribution of the fungus in the field.

MATERIALS AND METHODS

Cultures

The isolation of the present strains of *Sporidesmium bakeri* viz: strains C, BS, G and CL, is described by Thornton and Ross (1959). The high-sporing strain C has been disposed by the Commonwealth Mycological Institute, Kew, England, as *Sporidesmium bakeri* Syd. (Hughes 1953), I.M.I. Herbarium No. 74473. The strains were maintained on potato-glucose agar or potato-carrot extract agar media at *cà* 4°C.

Chemicals

The following chemicals were obtained from British Drug Houses Ltd.: all inorganic chemicals (ANALAR grade, except sodium molybdate); thiamin, calcium pantothenate, pyridoxine; D-glucose, sucrose, soluble starch (all ANALAR grade); ethanol; all aliphatic acids, except α-ketoglutaric and DL-malic acids; D-glucosamine hydrochloride, L-asparagine and peptone.

Casamino acids (vitamin free) were obtained from Difco Laboratories, mannitol from Eastman Kodak Co., pectin (40 grade), tannic acid (leviss B.P.) and urea (ANALAR grade) from Hopkin and Williams Ltd., and agar (standard grade) from Davis Gelatine (N.Z.) Ltd.

Xylan, prepared from beech wood (McDonald 1952) was kindly donated by Mr I. R. C. McDonald, Dominion Laboratory, Wellington.

A suspension of cellulose was prepared by macerating Whatman's No. 50 filter paper with water in a blender. This suspension gave no reaction for reducing sugars when heated with Fehling's reagent.

All other chemicals were obtained from L. Light and Co. Ltd.

Media

COMPOSITION OF MEDIA

(1) *Vegetable Extract Media:* A description of the preparation of potato-glucose agar and potato-carrot extract medium is given by Thornton and Ross (1959). In the preparation of the potato-carrot extract medium, however, considerable loss of liquid occurred during the extraction process and without a standardised procedure it was not possible to obtain reproducible results. The extract from potatoes (200 g) and carrots (200 g) was therefore made up, after boiling and filtration, to the original volume of fluid (1l). Unless otherwise stated, this diluted extract was used throughout this study.

(2) *Other Media:* The basal salts solution used for the experiments described in Tables 3–8 had the following composition: KH_2PO_4, 1·0g; KCl, 0·5g; $MgSO_4 \cdot 7H_2O$, 0·5g; $FeSO_4 \cdot 7H_2O$, 0·01g; distilled water, 1l. The concentrations of carbon and nitrogen compounds are given in the Tables.

In media containing nitrate nitrogen, the carbon compounds were usually added before sterilisation but in media containing other forms of nitrogen, carbon compounds were added after separate sterilisation.

The compositions of the mixtures of growth factors, trace metals and purines and pyrimidines added to some of the media were as follows: (a) *Growth factors:* thiamin, 1 ppm.; nicotinic acid, 1 ppm.; riboflavin, 1 ppm.; calcium pantothenate, 0·5 ppm.; pyridoxine, 0·5 ppm.; biotin, 0·2 ppm.; p-aminobenzoic acid, 0·1 ppm. (b) *Trace metals* (*cf.* Steinberg 1950): iron ($FeCl_3 \cdot 6H_2O$), 2·6 ppm; zinc ($ZnSO_4 \cdot 7H_2O$), 0·4 ppm; copper ($CuSO_4 \cdot 5H_2O$), 0·1 ppm.; manganese ($MnCl_2 \cdot 4H_2O$), 0·05 ppm.; molybdenum ($Na_2MoO_4 \cdot 2H_2O$), 0·04 ppm. (c) *Purines and Pyrimidines:* adenine, cytosine, guanine, hypoxanthine, thymine, and uracil were each added at a concentration of 5 ppm.

pH values of Media

Unless otherwise stated, pH values of the potato-carrot extract media were not adjusted before sterilisation. All other media were adjusted to pH 6·0 before sterilisation.

Distribution of Media

Liquid media (20 ml) were distributed in conical flasks (100 ml).

Inocula

Disks (6 mm diameter) of mycelium, cut from the edge of colonies grown usually on Czapek-Dox agar, were used as inocula. One disk was used for each flask and floated on the surface of the medium with the hyphal side uppermost.

Incubation

Unless otherwise stated, cultures were incubated in the dark at 24°C.

Cultures were usually examined after incubation for 7, 14, and 21 days and the maximum crop yields of mycelium were normally reached within this period. Yields were usually greatest and were recorded after incubation for 14 days. However, when yields of mycelial felts were at least 10% greater after incubation for either 7 or 21 days, these yields are indicated and recorded in the appropriate Tables.

Measurement of Growth

The effects of temperature and initial pH of medium on growth were assessed by measuring colony diameters. The diameter of the inoculum disk (6 mm) was subtracted from the total diameter of the colony grown from this inoculum.

Growth in most other experiments was measured by obtaining dry weights of mycelial felts from single or duplicate flasks. The mycelial felts were removed by filtration and washed with cold water. The agar inoculum disk was removed and the felts then dried overnight at 105°C. Very good agreement was obtained between duplicate flasks.

Chemical Tests

DETERMINATION OF pH

The pH values of culture fluids were determined with the glass electrode using a Beckman pH meter, Model G.

DETERMINATION OF NITROGEN CONTENT OF MYCELIAL FELTS

Growth on the cellulose medium was estimated by measuring the nitrogen content of filtered, washed mycelial felts. Results were corrected for the trace amounts of nitrogen present in the inocula and the cellulose itself. Nitrogen was determined by the Kjeldahl method. The digestion was made with sulphuric acid (50%, v./v.) containing selenium dioxide (1%), and the ammonia formed distilled in the apparatus of Parnas and Wagner (Niederl and Niederl 1942). The distillate was collected and subsequently titrated in boric acid (1% solution in ethanol, 20% v./v.) containing the mixed bromocresol green-methyl red indicator of Conway and O'Malley (1942).

DETERMINATION OF AROMATIC COMPOUNDS

A qualitative estimation of the metabolism of p-hydroxybenzaldehyde and vanillin was made by the addition of a few drops of an aqueous solution of 2:4-dinitrophenylhydrazine hydrochloride (Brady's reagent) to the culture fluids. Uninoculated media gave a dense orange turbidity, whereas media in which these substrates had been oxidised, with the disappearance of the carbonyl group, no longer gave a precipitate with this reagent.

RESULTS

Effect of Temperature on Growth

Growth of three strains of *Sporidesmium bakeri* was good between 16°C and 31·5°C and greatest at *ca* 24°C (Table 1). A higher temperature, however, appeared to be more favourable for the production of spores. Sporulation of strain C on potato-carrot extract agar and potato-glucose agar was very good both at 24°C and at 28°C. Spores were also produced by strains BS and G in greater quantities on incubation on potato-glucose agar at 28°C. Sporulation of these three strains still occurred at 31·5°C. Dr T. R. Vernon (pers. comm.) has found that growth and spore production of these strains were best between 25°C and 30°C with the optimum possibly at 27°C.

TABLE 1—Effect of Temperature on Growth

Medium	Incubation Temperature (°C)	Diameters of Colonies (mm) after Incubation for 4 Days		
		Strain C	Strain BS	Strain G
Potato-glucose agar	*ca.* 16°‡	11	13	12
	ca. 20°‡	21	22	22
	24°	31	34	34
	28°	31	16	15
Potato-carrot extract agar†	24°	33	35	33
	28°	33	16	16
	31·5°	22	18	12

†The amounts of potatoes and carrots extracted were 20 g/l.
‡Cultures incubated at room temperature.

On both potato-glucose agar and potato-carrot extract agar at 24°C or 28°C strain C produced grey-dark brown mycelium after incubation for 4 days. Sectoring of strain C sometimes occurred, particularly on the potato-carrot extract medium. Sectors grew less vigorously and were usually less deeply pigmented and contained fewer spores than the remainder of the colony. After incubation for 14 days on these media, strain BS produced a dull white-fawn mycelium without sectoring but with olive-grey pigmentation near the inoculum. Sectoring of the mycelial colony of strain G was sometimes noted after incubation for 14 days and the fawn-olive mycelium occurred as dense or sparse patches. Sectoring of strain CL was not observed although olive-grey bands of growth occurred in the fawn-coloured mycelial colony.

Effect of pH of Medium on Growth

Growth of *S. bakeri* was good over a wide range of pH of medium and there was no well-defined optimum (Table 2). In most of the present nutritional studies, media were used at *ca* pH 6·0.

TABLE 2—Effect of pH of Medium on Growth

Initial pH of Medium	Diameters of Colonies (mm) after Incubation for 72 h at 24°C on Potato-carrot Extract Medium		
	Strain C	Strain BS	Strain G
3·1	2	1	2
4·0	8	8	12
5·0	11	14	13
5·5	12	14	14
6·1	12	16	14
6·6	11	16	15
7·0	12	14	14
7·6	11	13	14
8·1	12	14	13
8·6	12	14	12

The results recorded are the means of duplicate flasks.

Ado (1946) used media, initial pH 6·4, for studying growth of *Sporidesmium* spp. which, after incubation for 1 month at 22°C–25°C, altered the pH of the medium to 6·8. Growth of the present strains of *S. bakeri* in potato-carrot extract medium resulted in a change to an alkaline pH of the medium.

Effect of Growth Factors on Growth

In Czapek-Dox and Raulin-Thom media, Thornton and Ross (1959) found that growth of *S. bakeri* strain CL was stimulated by the addition of yeast extract. In the present basal medium, the addition of yeast extract did not stimulate the growth of strain C, but did result in more rapid growth of strains BS and G. Similar results were obtained by replacing yeast extract with defined growth factors. On prolonged incubation, however, the crop yields of all strains were as great in the basal medium as in media containing yeast extract or growth factors (Table 3).

TABLE 3—Effect of Growth Factors, Trace Metals, and Purines and Pyrimidines on Growth

Medium	Crop Yields §,mg Dry Mycelial Felt/ml Culture Fluid		
	Strain C	Strain BS	Strain G
Basal medium†	7·5	8·7*	8·1*
Basal medium + growth factors	7·5	8·3	8·9*
Basal medium + growth factors + vitamin B_{12} (0·1 ppm.)	7·8*	8·0*	8·3
Basal medium + growth factors + trace metals	10·9	9·9	9·8
Basal medium + yeast extract (0·01%)	6·8	8·5*	7·4*
Basal medium + purines and pyrimidines	7·2*	7·6*	7·1*
Basal medium + nucleic acid (0·01%; from yeast)	5·8*	8·0*	6·5*
Potato-carrot extract‡(a)	11·0**	9·5**	11·2**
Potato-carrot extract (b)	4·8**	5·2**	5·8**

†*Composition of Basal Medium:* Basal salts solution + sodium nitrate (0·2%) + glucose (3·0%). The composition of the mixtures of growth factors, trace metals, and purines and pyrimidines are given in Materials and Methods.
‡*Potato-carrot extract Media:* (a) Potatoes and carrots (200 g each) were boiled with water for 30 minutes and the extract filtered. (b) The above extract was made up to the original volume of 1l.
§ * Incubation period, 21 days.
**Incubation period, 7 days.
All other cultures were incubated for 14 days.

The addition of purines and pyrimidines, or a small amount of nucleic acid, to the basal medium did not increase the crop yields of mycelial felts and, in general, growth was not stimulated by these compounds.

Effect of Trace Metals on Growth

The addition of trace metals to the basal salts-growth factor medium stimulated growth and resulted in increased production of mycelial felts. Although growth was slower in these synthetic media, crop yields were usually as great as those obtained in the more concentrated potato-carrot extract medium. The differences between yields in the potato-carrot extract media would be due to the different concentrations of the extracts (Table 3).

Sporulation of all strains in synthetic media was increased by the addition of trace metals. On most synthetic media, however, spore production was less than on the potato-carrot extract medium. It was observed that cultures of strain C in several synthetic media sporulated much more readily in flasks which had been contaminated with a large bacillus. Chemical factors encouraging sporulation of this fungus are not at present known.

Utilisation of Carbohydrates and Alcohols

The three strains of *S. bakeri* utilised many carbohydrates for growth and gave high crop yields of mycelial felts (Table 4). The intensity of the pigmentation of the mycelium of strain C, and the number of spores produced, generally depended on the suitability of the carbohydrate for growth. The pigmentation of strains BS and G differed on different carbohydrates but starch supported good growth and pigmentation of both strains.

TABLE 4—Utilisation of Carbohydrates and Alcohols

Substrate†		Crop Yields ‡, mg DryMycelial Felt/100 mg Carbohydrate or Alcohol Supplied		
		Strain C	Strain BS	Strain G
Pentoses	D-Arabinose	9*	39*	36
	D-Ribose	6*	12*	3
	D-Xylose	30*	36	33
Hexoses	D-Fructose	30	33	36
	D-Galactose	24*	36	36*
	D-Glucose	33	33	36
	D-Mannose	33*	36	36
Disaccharides	D-Cellobiose	39	39	42
	Sucrose	36*	42	39
	$\alpha,\alpha,'$ D-Trehalose	9	48	42
Polysaccharides	Hemicellulose	9	6	9
	Xylan	12	20	19
	Pectin	27	27	30
	Starch	30*	36	33*
Alcohols	Ethanol	1*	3*	5
	Glycerol	1*	1*	1*
	i-Inositol	3*	5	10*
	Mannitol	6*	10*	10*

†*Composition of Culture Media:* Basal salts solution + sodium nitrate (0·2%) + trace metals + carbohydrate or alcohol substrate (3%).

‡Crop yields were recorded after incubation for 14 days, except for those marked with an asterisk where the yields were recorded after incubation for 21 days.

Strain C differed from the other two strains in its growth on arabinose and trehalose, and strain BS differed in its growth on ribose. The other eleven carbohydrates supported similar crop yields of the three strains.

Growth of the three strains on the alcohols tested was generally poor although moderate growth was obtained with mannitol as substrate.

Utilisation of Cellulose

The three strains of *S. bakeri* could utilise cellulose as sole carbohydrate and the extent of growth with this substrate was assessed by determining the nitrogen content of the mycelial felts (Table 5). Although the mycelium of the three strains spread over the surface of the culture medium, growth was not vigorous and a considerable amount of the cellulose remained undigested after incubation for 63 days. After this incubation period, the dark grey-black mycelial felt of strain C contained many spores and the white-fawn felts of strains BS and G contained several black, spore-containing areas.

TABLE 5—Utilisation of Cellulose

Incubation Time (Days)	Nitrogen Content of Mycelial Felts, mg/flask		
	Strain C	Strain BS	Strain G
28	1·37	1·35	1·39
63	1·97	1·69	1·97

Composition of Culture Medium: Basal salts solution + sodium nitrate (0·2%) + cellulose (1·0%) + trace metals. This medium contained 6·59 mg nitrogen/flask.

Utilisation of Aliphatic Acids

Although strain BS did not appear to grow on a citric acid medium or strain C on a lactic acid medium, the three strains of *S. bakeri* generally showed similar ability to utilise aliphatic acids (Table 6). Most of the acids were utilised only to a slight extent and only with acetic acid or succinic acid did crop yields of mycelium approach those found on the carbohydrate media. Growth on all media was too sparse for many spores to be produced.

TABLE 6—Utilisation of Aliphatic Acids

Acid Substrate†	Crop Yields‡, mg Dry Mycelial Felt/100 mg Acid Supplied		
	Strain C	Strain BS	Strain G
None	2*	2	1
Acetic	10	16*	9
Citric	4*	2	8*
Fumaric	5*	4*	4
α-Ketoglutaric	4	9*	8
Lactic	2*	5*	4
DL-Malic	4*	6	4
Succinic	8*	16*	13
Tartaric	1	1	1

†*Composition of Culture Media:* Basal salts solution + aliphatic acid (0·5%) + trace metals. Media were adjusted to pH 6·0 with ammonia which served as the sole source of added nitrogen.
‡Crop yields were recorded after incubation for 14 days, except for those marked with an asterisk where the yields were recorded after incubation for 21 days.

Utilisation of Aromatic Aldehydes and Acids

All strains completely utilised p-hydroxybenzaldehyde after incubation for 21 days but growth of strain BS was little different from that on medium without added carbon substrate (Table 7). Vanillin was almost completely utilised by strain BS after incubation for 21 days but a slight precipitate with Brady's reagent was still given by the culture fluids of the other strains. Growth of strain G on the vanillin medium was similar to that on medium without added carbon substrate. The three strains grew on the ferulic acid medium and produced, after incubation for 21 days, orange-yellow culture fluids. These fluids probably contained ketones as they gave a very slight precipitate with Brady's reagent and also a deepening of colour on the addition of concentrated ammonia. Ferulic acid was, therefore, metabolised by this organism.

TABLE 7—Utilisation of Aromatic Compounds

Substrate†	Qualitative Assessment of Growth‡		
	Strain C	Strain BS	Strain G
p-Hydroxybenzaldehyde	+++	++	+++
Vanillin	++	++	+
Ferulic acid	++	++	++
Gallic acid	+	++	+
Tannic acid	+	+	+

†*Composition of Media:* Basal salts solution + sodium nitrate (0·2%) + trace metals + (a) p-hydroxybenzaldehyde, vanillin or ferulic acid (0·01%), or (b) gallic acid or tannic acid (0·1%). The p-hydroxybenzaldehyde, vanillin and ferulic acid were dissolved in sterile water and added, without sterilisation, to the separately sterilised basal medium. No contamination was observed in uninoculated media after incubation for 21 days.
‡ +++ indicates growth of the fungus and complete utilisation of substrate.
++ indicates growth of the fungus or complete utilisation of substrate.
+ indicates some utilisation of substrate but growth of the fungus no greater than in a control medium without the aromatic compounds.

All three strains grew poorly on gallic acid and tannic acid media, although strain BS showed some increase of yield on the gallic acid medium as compared with the carbon-free medium. After incubation for 21 days, however, the culture fluids of all strains were a very deep brown colour indicating that some metabolism of these acids had occurred.

Utilisation of Nitrogen Compounds

It is apparent from Table 8 that *S. bakeri* could use many inorganic or organic nitrogen compounds for growth and that the three strains had similar properties, except for their utilisation of chitin.

TABLE 8—Utilisation of Nitrogen Compounds

Nitrogen Substrate†	Crop Yields‡, mg Dry Mycelial Felt/100 mg Glucose Supplied		
	Strain C	Strain BS	Strain G
Sodium nitrate§	25*	33*	31*
Sodium nitrate	33*	31	38*
Ammonium phosphate§	11	17	17
Urea	31*	43	29
D-Glucosamine	28*	49*	43*
γ-Amino-n-butyric acid	42*	49*	47*
L-Asparagine	40	45*	45*
L-Aspartic acid	43*	44*	48*
L-Glutamic acid	48	39*	40*
L-Glutamine	15	19	19*
Glycine	46*	51*	48*
Casamino acids§	34	35*	33
Peptone	31*	41*	41*
Nucleic acids (from yeast)	28*	48*	46*
Chitin	9*	31*	33*

†*Composition of Culture Media:* Basal salts solution + glucose (3·0%) + nitrogen substrate (0·0167% N; i.e. equivalent to 0.1% $NaNO_3$) + trace metals, except for those substrates marked § where no trace metals were added.
‡Crop yields were recorded after incubation for 14 days, except for those marked with an asterisk where the yields were recorded after incubation for 21 days.

Utilisation of ammonia caused the development of acidity in the medium and, after incubation for 14 days, the pH was usually between 2·6–2·8. Crop yields were, therefore, rather low in the ammonium medium. In the nitrate media, pH values were about 6·6 after growth for the same incubation period.

In several of the media with organic nitrogen compounds, a considerable amount of submerged mycelium was produced and this rather mucoid growth was difficult to filter and wash. The crop yields recorded in Table 8 for many of the organic nitrogen substrates may, therefore, be a little high due to residual salts or glucose in the washed mycelial felts.

Urea was readily utilised by the present strains and growth with glucosamine was good. Considerable acidity was produced in the medium with glutamine as sole source of nitrogen and pH values of the culture fluids were 3·2–3·4 after incubation for 14 days. Due probably to this acidity, the mycelial felts did not spread over the entire surface of the glutamine medium and crop yields were low. Amino acids and asparagine supported good growth of all strains. After growth in the presence of glutamic acid, the culture fluids were between pH 5·0 and 5·8. With the other defined organic nitrogen compounds, the culture fluids were between pH 6·0–6·7 after the incubation periods indicated in Table 8.

Chitin was poorly utilised by strain C but the other complex nitrogen compounds supported good crop yields and produced culture fluids usually between pH 4·7–5·6.

Growth on potato-carrot extract medium was examined as part of this experiment. Crop yields were recorded after incubation for 7 days as they were less after incubation for 14 days. The mean crop yields of the three strains, expressed as mg dry mycelial felt/ml culture fluid, with the ranges of values in parentheses, were as follows: (a) on potato-carrot extract medium, 5·3 (4·8–5·8); (b) on sodium nitrate medium, 10·2 (8·2–11·5); (c) on amino acid media (γ-amino-n-butyric acid to glycine, inclusive, as listed in Table 8), 12·0 (4·4–15·2).

Although yields of mycelial felts on the defined synthetic media compared very favourably with those on the potato-carrot extract medium, sporulation was greatest on the potato-carrot medium and was greater on this medium than on the other media containing complex nitrogen compounds. Sporulation by the fawn-dark grey mycelium of strain C was, however, very good on the γ-amino-n-butyric acid and asparagine media and glycine and nitrate also supported moderate spore production. After incubation for 21 days, strains BS and G developed dark brown patches on the fawn mycelia, particularly in the glycine medium, but spores were not seen on microscopic examination.

DISCUSSION

The present strains of *S. bakeri* differed considerably in their ability to produce spores. Spores were produced only rarely or in low numbers by strains CL, BS and G but were produced by strain C, often in high numbers, on both non-defined and synthetic media. Sporulation was heaviest on non-defined media, particularly on potato-carrot extract. The chemical factors encouraging sporulation are at present unknown. Sporulation of strain C

was, however, favoured by the presence of γ-amino-n-butyric acid or asparagine in a defined medium and it is possible that other nitrogen compounds may also be effective in promoting spore production. The different carbohydrates tested did not have any marked effect on sporulation.

Although the present strains differed considerably in their production of spores, they generally exhibited similar responses to different temperatures and pH of media and also similar nutritional behaviour. Added growth factors were not essential for growth although they may have been stimulatory for some strains. The fungus could utilise many carbohydrates, alcohols, and aromatic compounds and could obtain its nitrogen from many inorganic and organic nitrogen compounds. The fungus readily utilised pectin, and hemicellulose was also attacked. Cellulose supported moderate growth of the fungus. The fungus, therefore, has simple growth requirements and a marked ability to utilise a wide variety of substrates for growth and it is unlikely that a shortage of nutrients would be a major factor limiting its distribution in the field. The effect of nutrients on toxin production, however, has yet to be determined.

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FACTORS INFLUENCING THE DISTRIBUTION PATTERN OF PLANKTON IN THE MIXING ZONE BETWEEN OCEANIC AND HARBOUR WATERS

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Summary

Using a pumping technique, previously described, 60 plankton samples were taken in a continuous series at a depth of 5 metres. The line of samples covered a distance of about 4 miles, running from Cook Strait up the main entrance channel of Port Nicholson. Temperature and salinity were recorded simultaneously from the same depth. The water traversed is divided into three zones: 1. Cook Strait; 2. intermediate mixing zone; 3. harbour water. Six dominant species of plankton were counted in the samples. For each zone, multiple regression equations are computed, relating numerical abundance of each species to that of the remaining 5 species, and to temperature, and salinity.

The partial regressions of *Oithona similis, Elminius modestus* nauplii, *Polydora polybranchiata* larvae, and *Coscinodiscus wailesii,* were positive on temperature, but negative on salinity. The partial regression of *Paracalanus parvus* was negative on temperature, positive on salinity. It is possible that the combination of two opposite-sign regressions may be induced by density effects. Both *Paracalanus* and *Elminius* showed the same relationship to temperature and salinity as in a previous experiment. *Oithona* and *Elminius* both showed positive partial correlation to *Paracalanus* but not to each other. Neither of these correlations agrees with previous experiments and it is possible that other variates, as yet unknown, are involved in the relationship. Negative partial correlations between species do not reach significance but special attention has been paid to such relationships owing to their apparent rarity. It is possible that *Coscinodiscus* is inversely related to both *Paracalanus* and *Melosira granulata.* Otherwise *Melosira* is entirely independent of both other species and physical variates.

The results of this and previous experiments are discussed. Factors influencing spatial distribution of plankton species may be divided into four categories: (1) random variation; (2) variable physical properties of the water; (3) other pelagic species; (4) internal behaviour patterns between individuals of the same species.

INTRODUCTION

The experiment to be described is one of a series which has been designed to elucidate factors responsible for the pattern of distribution of plankton organisms on a relatively small spatial scale (that is, as shown by samples taken at intervals of 100 metres or less). The problem becomes apparent from the observation that replicate plankton samples, even when place, time, and technique are carefully standardised, almost invariably yield quantitative population estimates which differ far more than would be expected from errors due to chance in a randomly distributed population. In ecological plankton investigations, excess variability is commonly regarded as a nuisance to be avoided or minimised. In the present work the aim is to utilise this nuisance as a source of information concerning behaviour patterns of the

plankton community. Previous contributions have been made to the same subject (Cassie, 1957, 1958, 1959a, 1959b). It has been suggested (Cassie 1957) that an overdispersed distribution of the discrete log-normal type may arise owing to the relative abundance of plankton being geometrically related to variations in physical properties of the water.

The apparatus used in the present experiment has been described previously (Cassie 1958). It consists of a centrifugal pump, suction hose and plankton filter which may be employed to take a series of 200 litre plankton samples continuously at 1 min intervals in either a vertical or a horizontal series. Electronic sensing circuits make a continuous record of temperature and salinity of the water before it enters the pump.

An earlier series of samples in a horizontal plane was taken at a depth of about 10 metres within Wellington Harbour, where there is a complex and turbulent mixing of waters of different properties under the influence of a confined tidal circulation (Cassie 1959a). In the work to be discussed the principal object was to extend the earlier results (Cassie 1959a) by sampling in a similar manner in an adjacent area where a somewhat different mixing pattern is present in the water. A single horizontal line of samples was taken, extending from the open waters of Cook Strait into the entrance channel leading to Port Nicholson. The pattern of mixing between the oceanic and harbour waters is simpler and less turbulent than that commonly found within the harbour, with only one major discontinuity which is of a less abrupt type. A secondary aim of the change in location was to obtain some indication of the causes underlying the change in plankton population between oceanic and inshore waters. The results confirm and extend some of the conclusions from the previous experiment.

Sampling Procedures

Samples were collected on 28 September 1958, between 1400 and 1500 hr. The weather was fine and clear with a light northerly breeze which had flattened the swell, following a strong southerly blow two days previously. Seas were slight, and low cloud cover was about 3/10. The apparatus was mounted aft on the quarterdeck of RNZFA *Tui*, the hose intake and depressor suspended by the main winch wire from a fairlead over the stern. Sufficient wire was veered to maintain the intake at a depth of 5 metres at towing speed. The towing run commenced with Pencarrow Head abeam and proceeded in a northerly direction at 4·3 knots (ground speed) along the main navigational channel. The course was plotted from radar fixes at 10 min intervals (circles in Fig. 1). A total of 60 samples was taken at 1 min intervals, the beginning and end of each sample being indicated by dots along the track line. Total distance covered was 4·3 nautical miles (8·0 kilometres) and the average length of each sample was 133 metres. Since the ship was travelling in the same direction as a tide stream of about 0·8 knots, the actual horizontal cylinder of water sampled was about 110 metres per sample, which is almost the same length as in the previous samples (Cassie 1959a). In addition to the zooplankton samples, each filtered from 200 litres of water, a 300 ml sub-sample of unfiltered water was taken with each main sample for subsequent extraction of phytoplankton.

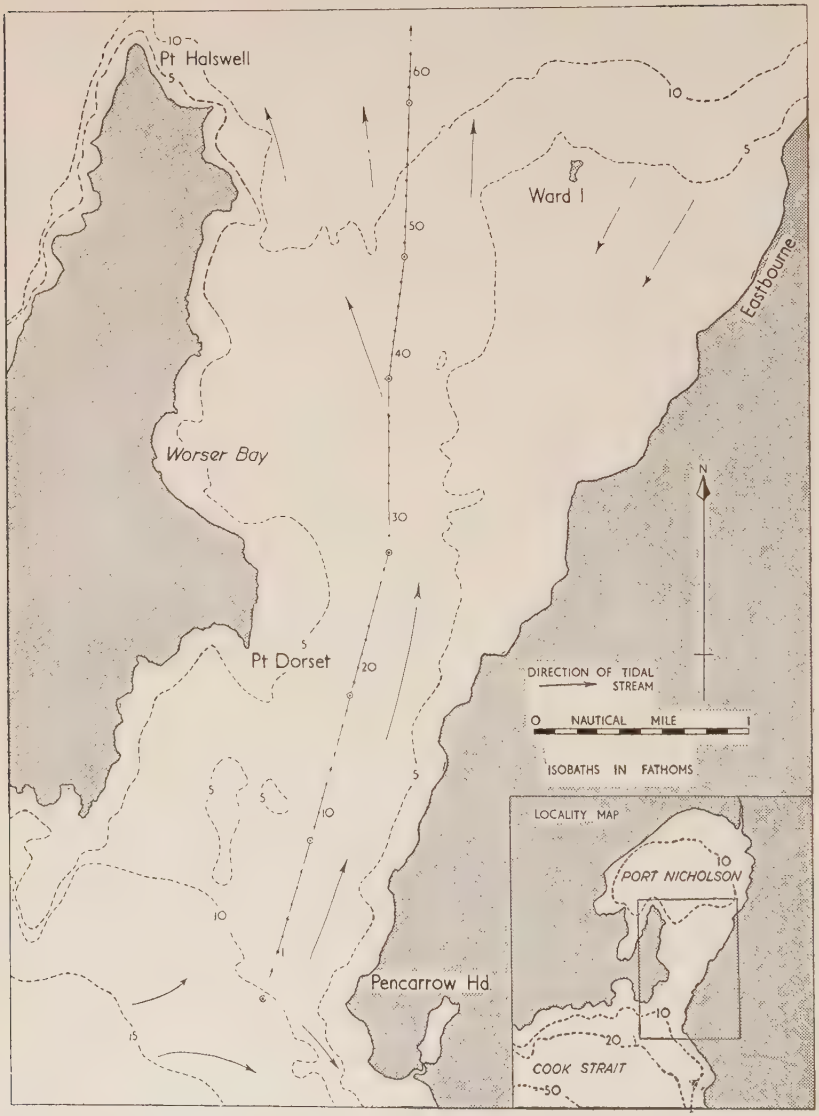

FIG. 1—The Entrance Channel, Port Nicholson, showing course of ship and position of samples. Dots: beginning and end of sample. Circled dots: radar fixes. Samples numbered at point of commencement.

Zooplankton samples were completely enumerated by standard methods. Phytoplankton subsamples were concentrated with a Foerst continuous centrifuge and counted in a modified Sedgwick-Rafter cell. This cell was of standard horizontal dimensions, but of greater than standard depth (2·5 mm), thus having its capacity increased sufficiently to accommodate

the entire sample. Some change in the proportion of the sample counted was made accordingly to the varying abundance of phytoplankton, but at least $\frac{1}{8}$ of the total volume was scanned and counted in each sample. Appropriate correction factors were employed to obtain an estimate of the total numbers. Preliminary tests indicated that the variance of estimates was two to three times as great as Poisson expectation, but was still small in relation to the sample variance. The error introduced is not sufficient to affect the validity of conclusions.

PHYSICAL CHARACTERISTICS OF THE WATER

Predicted high tide for Wellington was at 1718 hr so that the tide stream was moving northward at the time (1400–1500 hr) at a mean speed of 0·8 to 0·9 knots (Chart NZ50). Tide arrows are as indicated for flood tide by Brodie (1958). A deliberate change in course was made at the fourth fix (just south of sample 30) but a slight flexure in the track indicated by the fifth fix has been produced by the westward component of the tide stream about this position.

The temperature and salinity record is shown at the top of Fig. 3. The variation of both these properties within any one sample was small so that

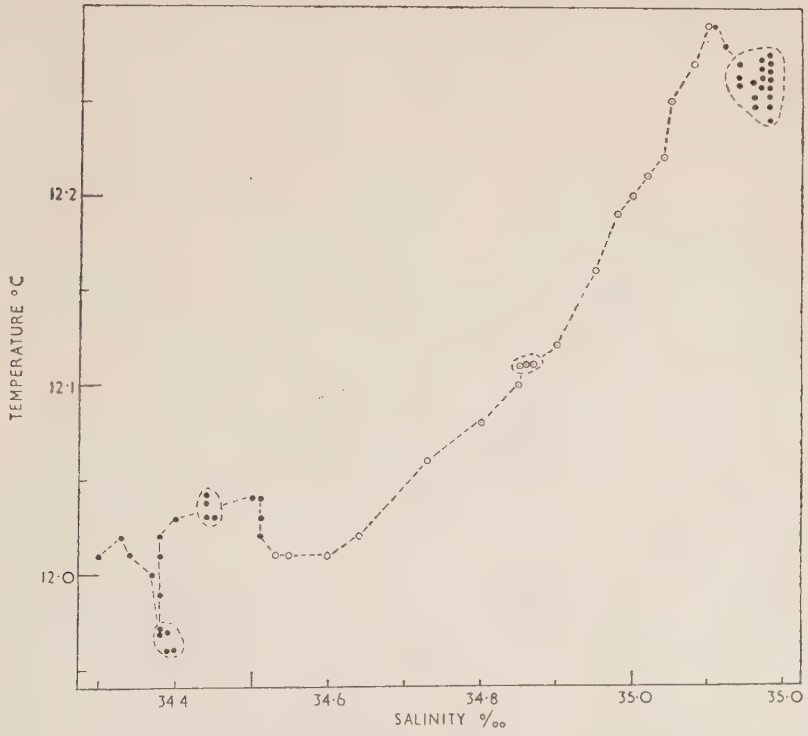

FIG. 2—Temperature-salinity (T-S) Diagram for Samples 1–60. Black circles: zones 1 and 3. White circles: zone 2. Broken line joins samples in order of occurrence.

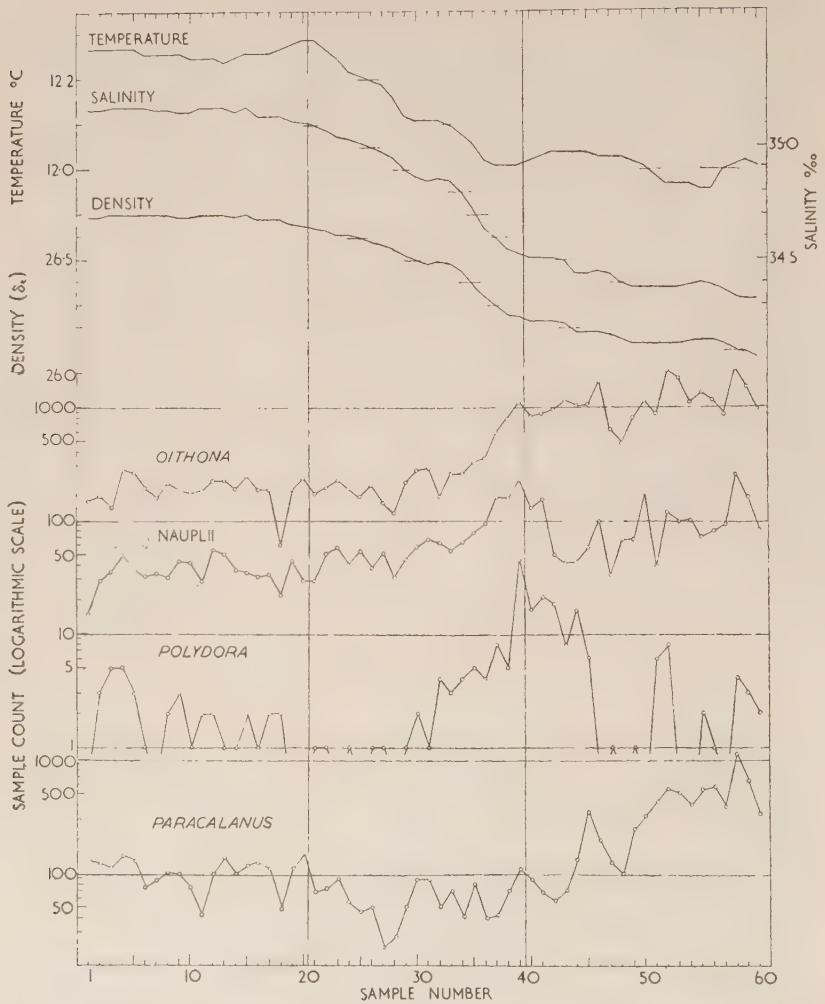

FIG 3—Temperature, salinity density and sample counts for zooplankton (sample counts on log. scale).

the linear interpolation beween means for each minute is a faithful representation of the original record within the limits of accuracy of the recorder. This result is in strong contrast to that of a previous experiment (Cassie 1959a) where oscillations with a range up to 0·4°C and 0·5% salinity occur within the longitudinal range of some samples. The difference may be in part responsible for the stronger correlations which have been found in the plankton : temperature-salinity analysis in the present experiment. The general pattern of temperature and salinity is relatively simple, and samples may be allocated to three zones, each roughly a third of the entire sampling

run. The southern zone (1) is relatively isothermal and isohaline and represents undiluted oceanic water from Cook Strait which has penetrated slightly over 1 mile into the harbour with the incoming tide. The middle zone (2) represents an area of mixing between oceanic and harbour water. Both temperature and salinity fall rapidly but relatively smoothly in a northward direction, again a contrast to the turbulent mixing pattern found previously in the inner harbour. The northern zone (3), though slightly less homogeneous than zone 1, again shows levelling out of the physical properties. This represents harbour water approaching the last stage in its gradual movement toward and mixing with oceanic waters, the low temperature, low salinity source being continuously replenished from the Hutt River and other smaller streams.

The T-S (temperature-salinity) diagram (Fig. 2) has been used as a basis for dividing the traverse into Zones 1, 2, and 3 containing samples 1–20, 21–39, and 40–60 respectively. In Zone 2 there is a strong positive correlation between temperature and salinity ($r = 0{\cdot}97$). In the other two zones the correlation is less pronounced though significant in both cases, negative ($r = -0{\cdot}55$) for Zone 1 and positive ($r = 0{\cdot}48$) for Zone 3. Density, (δ_t), is also plotted in Fig. 3. This curve follows the trend of salinity very closely, indicating that temperature (which would influence density in the opposite direction) has been a relatively minor factor in determining its variation.

THE PLANKTON

The following plankton species were present in sufficient numbers to be considered in the statistical analysis:

Copepoda	*Paracalanus parvus*	*P*
	Oithona similis	*O*
Cirripedia	*Elminius modestus*, Stage V and VI nauplii	*N*
Polychaeta	*Polydora polybranchiata*, late larvae	*P*
Diatomales	*Coscinodiscus wailesii*	*C*
	Melosira granulata, var. *augustissima*	*M*

The initial letter of the generic name will be used as the symbol for the species in equations and certain tables. The diatom, *Coscinodiscus wailesii* was large enough to be taken by the zooplankton filter, in which it occurred in considerable numbers. The same species was relatively rare in centrifuged subsamples owing to the smaller water volume. However, on a biomass basis, this species was probably the dominant phytoplankton species and could be considered at least as significant a member of the biotic complex as the more numerically abundant *Melosira*. The refractile nature of the latter species made it readily distinguishable in the centrifuged material in spite of the presence of large quantities of suspended organic matter, but the small size of individual cells rendered impracticable any count other than of entire chains. The number of cells per chain ranged (approximately) from 1 to 20, with a mean of about 5.

The following species were also identified as being present in the majority of samples.

Copepoda	*Calanus finmarchicus* Brodsky
	Corycaeus aucklandicus
Decapoda	Zoaea and megalopa larvae
Diatomales	*Coscinodiscus centralis*
	Nitzschia closterium
	Rhizosolenia setigera
	Asterionella gracillinia
	Synedra fulgeus
	Thalassiothrix nitzschioides

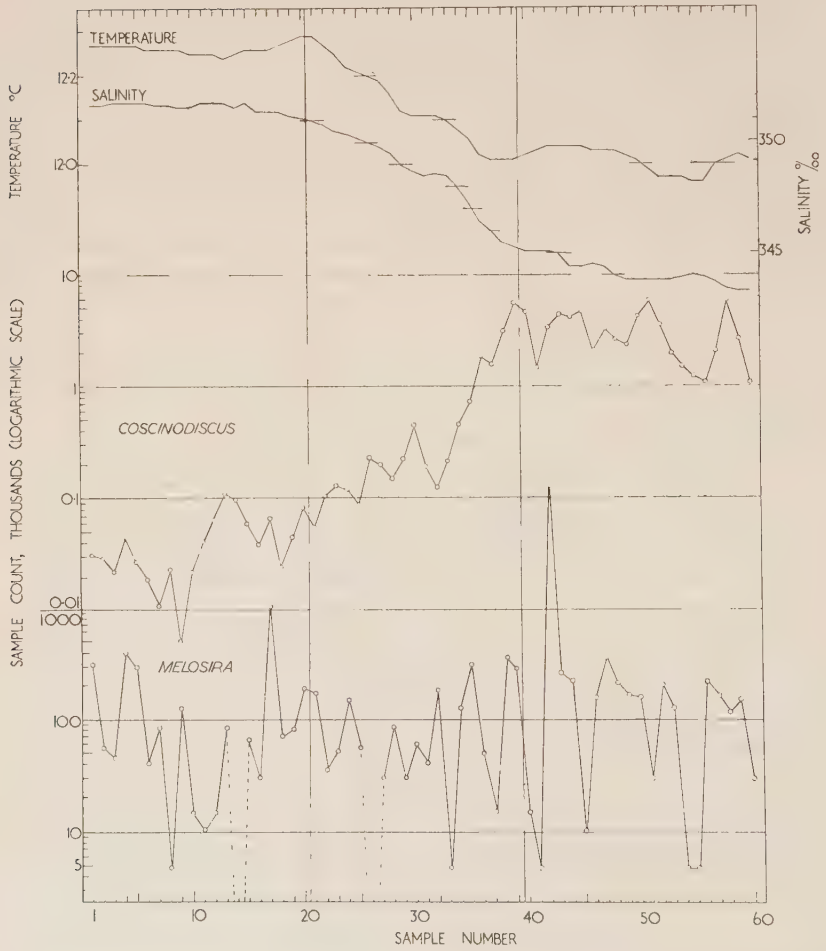

FIG. 4—Temperature, salinity and sample counts for phytoplankton (sample counts on log. scale).

Nitzschia was also abundant (several hundred) in a few isolated subsamples, but the number of zero counts was too great for it to be used in the statistical analysis. There was a complete absence of *Nitzschia* in samples 1–14, but in each of two samples taken immediately before the commencement of the recorded samples the number of individuals present was about 1,500.

The numbers of the four zooplankton species are plotted in Fig. 3, and of the two diatoms in Fig. 4, the scale being logarithmic in both. *Melosira* counts are reported as numbers of chains and are converted to a basis comparable to that of other species by multiplying by 600 (200 litres/330 ml). Some of the more pronounced correlations which will be revealed in the regression analysis are already evident in the graphical presentation. It is evident that *Oithona, Elminius* nauplii and *Coscinodiscus* are inversely related to the physical properties, while the similarity between the *Oithona* and nauplii curves is particularly close.

TABLE 1—Means, Variances, and Coefficients of Dispersion (Untransformed Data)

	Paracalanus			*Oithona*			*Elminius*		
Zone	1	2	3	1	2	3	1	2	3
$\bar{x}$	107	62	349	191	317	1,167	36	74	96
s^2	829	548	68,040	2,409	61,860	209,300	92	2,583	2,418
c	0·06	0·12	0·55	0·06	0·61	0·15	0·04	0·46	0·23
d	0·87	0·89	0·995	0·92	0·995	0·995	0·61	0·97	0·96

	Polydora			*Coscinodiscus*			*Melosira*		
Zone	1	2	3	1	2	3	1	2	3
$\bar{x}$	1·8	4·5	5·4	44	837	3,100	238	181	811
s^2	2·2	96·0	45·2	835	2,067,000	2,368,000	111,100	32,910	7,511,000
c	0·11	4·47	1·38	0·42	0·29	0·25	1·96	1·01	11·43
d	0·17	0·95	0·88	0·95	0·96	0·999	0·998	0·995	0·9999

Table 1 gives the four statistics, $\bar{x}$, s^2, c and d for each of the six species in the three zones. $\bar{x}$ and s^2 are the arithmetic mean and variance, while c and d are indices of dispersion defined by the equations:

$$c = (s^2 - \bar{x})/\bar{x}^2$$
$$d = (s^2 - \bar{x})/s^2$$

(Cassie 1959a, 1959b).

In general the mean number of individuals, $\bar{x}$, per sample is greatest in zone 3 (harbour water), least in zone 1 (oceanic water) and intermediate in value in zone 2. However, both *Paracalanus* and *Melosira* depart from the general rule in that the ranking of zones 1 and 2 has been interchanged. It will be seen in the regression analysis that these two species also differ from the other four in their reactions to temperature and salinity, a fact which may help to account for the discrepancy in means.

There is a large excess of variance over mean in every species except in *Polydora* (zone 1), indicating without doubt that the distribution is non-random. The proportion of variance in excess of Poisson is shown by d to be very high, greater than 0·99 in seven instances so that the contribution of chance in determining the spatial pattern is negligible. The index of dispersion, c, gives some measure of the relative level of overdispersion (independent of abundance) in each group. It might perhaps be anticipated that overdispersion would be least in the relatively homogeneous oceanic water (zone 1), somewhat greater in the more heterogeneous harbour water (zone 3), and greatest in the mixing zone (zone 2) between the two. This is exactly the situation with *Oithona, Elminius* and *Polydora* but the remaining three species do not fit this hypothesis so well. *Melosira* in particular appears to have a pattern which is very little dependent upon the physical properties, a conclusion which is further supported by the regression analysis.

STATISTICAL METHODS

The statistical analysis is in the form of multiple regression analysis in which each of the six species of plankton in turn is made the dependent variate, the remaining five species, plus temperature and salinity being the independent variates. Independent variates with non-significant ($P > 0{\cdot}05$) partial regression coefficients are eliminated from each equation, except in a few instances where there is other evidence to support the reality of the relationship. Each zone is treated separately so that there are in all 18 equations. Five of these, however, are eliminated owing to the absence of any but zero order coefficients.

No attention has been paid to autoregression within species since it has been shown (Cassie 1959a, 1959c) that there is little or no gain in information from so doing, even though autoregression may sometimes remain significant after being made partial on other factors. It is thus scarcely likely that the considerable increase in computation would have been profitable in the treatment of present data.

Throughout the regression and correlation computations logarithmic values of plankton counts have been used, only temperature and salinity being untransformed. This is a departure from previous procedure (Cassie 1959a, 1959c) where untransformed numbers were used for species on species regression. The new method has the advantage that both species: species, and species: physical-variable regressions are stictly compatible, the least squares estimate in both cases being determined from the logarithms of species. There does not appear to be any *a priori* preference for transformed or untransformed data since either may be more effective in revealing correlation under different circumstances. However, if it is assumed that an interspecific correlation is the result of the two species having a common reaction to an external (physical) factor rather than to each other, the log. transformation may be more appropriate. It has been shown (Cassie 1959a) that the numerical relationship of an organism to a physical variable is commonly

geometric so that if species Y_1 and Y_2 are dependent upon the factor X, the two regressions may be expressed as:

$$\log Y_1 = a_1 + b_1 X$$
$$\log Y_2 = a_2 + b_2 X$$
Eliminating X: $\log Y_1 = a + b \log Y_2$
(where $b = b_1/b_2$, $a = a_2 b - a_1$)

This last equation is identical with that employed in the present paper for interspecific relationships.

In Table 2, linear correlation coefficients for the four zooplankton species are given in brackets for comparison with log-values. In the majority of correlations the two coefficients are sufficiently similar in value to have no significant effect upon the ultimate analysis. It can be assumed, then, that the regressions are approximately linear with zero intercept. In five cases, the significance level has been affected by the transformation, but invariably the log correlations give greater consistency with other zones. The two correlations (zone 1, *Paracalanus:Oithona* and *Oithona:Elminius*) which gain significance are much more drastically changed than the three (zone 2, *Paracalanus:Elminius*; zone 3, *Paracalanus:Polydora* and *Paracalanus: Oithona*) which lose significance so that on the aggregate there has been gain in information from the use of logarithms.

Computations for species counts were carried out on three figure logarithms, the raw data being considered accurate to two figures for the most part. In the few instances where zero sample counts occur, these are transformed as log 0·5 (solitary zeros) or log 0·25 (two adjacent zeros). For the initial total correlations, six significant figures were carried, although only two are given in Table 3. This enables between three- and four-figure accuracy to be maintained in the regression analysis, although here again only two decimal places are reported since it appears that order of magnitude and statistical significance are of more importance than absolute values of coefficients.

Each multiple regression equation is initially computed as a least squares estimate of the general form:

$$\log \hat{Y} = a + b \log X + \ldots\ldots\ldots + b' Z + \ldots\ldots\ldots$$

Where $\hat{Y}$ is the predicted number of individuals of species Y in the presence of X individuals of species X and a value Z of the physical property Z; a is a constant, b and b' are partial regression coefficients.

It is, however, more convenient to express the relationship in the form:

$$\hat{Y} = \bar{y}\, x^{b} \ldots\ldots\ldots B'^{z} \ldots\ldots\ldots$$

Where y is the mean value of Y, x and z are deviations of X and Z from their respective means, $B' = \text{antilog } b'$. This implies that Y increases as the bth power of X. When the relationship is one of simple proportion b will approximate to unity. For unit increase of Z, Y will increase to B' times

TABLE 2—Means, Standard Deviations, and Total Correlation Coefficients

(Species Transformed to Logs. Linear Correlations in Brackets)

Zone		*T*	*S*	Log *P*	Log *O*	Log *N*	Log *P′*	Log *C*	Log *M*
1.	$\bar{x}$	12·26	35·16	1·01	1·26	0·54	0·11	1·54	2·90
	s	0·012	0·021	0·14	0·14	0·13	0·037	0·32	0·89
	T		− 0·55	0·32	− 0·12	− 0·36	− 0·23	− 0·10	0·46*
	S			− 0·09	0·20	0·17	0·60**	− 0·16	− 0.13
log.	*P*				0·58**(0·19)	0·20(0·18)	− 0·09(0·01)	0·30	0·32
log.	*O*					0·54*(0·17)	− 0·00(0·01)	0·34	0·03
log.	*N*						0·22(0·12)	0·12	− 0·14
log.	*P′*							− 0·24	0·01
log.	*C*								− 0·00
2.	$\bar{x}$	12·13	34·86	0·76	1·42	0·80	0·13	2·51	2·91
	s	0·091	0·180	0·18	0·25	0·24	0·050	0·57	0·84
	T		0·97***	− 0·07	− 0·75***	− 0·80***	− 0·80***	− 0·87***	− 0·20
	S			− 0·16	− 0·86***	− 0·90***	− 0·87***	− 0·95***	− 0·26
log.	*P*				0·50*(0·49*)	0·32(0·39†)	0·31(0·48*)	0·19	0·18
log.	*O*					0·93***(0·97***)	− 0·83***(0·84***)	0·91***	0·21
log.	*N*						0·84***(0·82***)	0·88***	0·30
log.	*P′*							0·84***	0·27
log.	*C*								0·19
3.	$\bar{x}$	12·01	34·41	1·41	2·04	0·93	0·14	3·43	3·16
	s	0·028	0·060	0·37	0·17	0·21	0·20	0·24	0·77
	T		0·48*	− 0·07	− 0·40	− 0·17	0·13	0·45*	0·31
	S			− 0·83***	− 0·28	− 0·33	0·16	0·23	0·07
log.	*P*				0·61**(0·71***)	0·40(0·62**)	− 0·12(− 0·41†)	− 0·14	− 0·02
log.	*O*					0·58**(61**)	0·00(− 0·14)	0·03	− 0·00
log.	*N*						− 0·02(− 0·04)	− 0·04	− 0·02
log.	*P′*							0·13	0·00
log.	*C*								0·03

its previous value. When B' is negative it is convenient to report the reciprocal B'^{-1}, thus giving the exponent Z a negative sign, i.e. Y will decrease to $1/B'$ of its previous value for unit increase of Z.

As in previous work (Cassie 1959a) it has been convenient to express Z (etc.) in the units 0·1°C and 0·1‰ salinity in computing regression coefficients since the entire sampling range does not cover more than one unit of temperature or salinity.

For each regression equation a multiple correlation coefficient R is computed. This is reported as the square R^2, which gives a measure of the proportion of the variances of the dependent variable species which has been accounted for by regression. Levels of significance of regression coefficients are shown as follows:

$$\begin{aligned} \dagger,\ & P < 0{\cdot}1 \\ *,\ & P < 0{\cdot}05 \\ **,\ & P < 0{\cdot}01 \\ ***,\ & P < 0{\cdot}001 \end{aligned}$$

As an example, the regression equation for *Oithona* in zone 2 may be written in full (from Table 3):

$$\hat{O} = \bar{o}\ 1{\cdot}64^{*t}1{\cdot}53^{***-s}p^{0.36**}n^{0.29},\ R^2 = 0{\cdot}95$$

Where o is the mean sample count of *Oithona*, $\hat{O}$ is the predicted sample count at $(T + t/10)$°C, $(S - s/10)$‰ salinity and when $(P + p)$ *Paracalanus* and $(N + n)$ *Elminius* are present.

Results of Regression Analysis

Table 2 summarises the means, $\bar{x}$, standard deviations, s, and total correlation coefficients from the log.-transformed species counts, and from linear values of temperature and salinity. s may also be regarded as an index of dispersion, and, as with c and d (Table 1) there is a tendency for highest values to occur in zone 2 and lowest in zone 1, although again consistency is not complete.

Owing to the complex nature of the relationships concerned, it is not valid to draw any definite conclusions from the total correlation coefficients, which are but an intermediate step in processing the raw data. However, the same relationships are found which have already been discussed in connection with the graphical presentation (Figs. 3 and 4). In zones 1 and 3 the standard deviations of temperature and salinity are low, and in fact verge on the limits of accuracy of the *T-S* recorder, so that it is doubtful whether any correlations with these properties will be valid even though some may be statistically significant. In zone 1, temperature and salinity correlations have been ignored in the regression analysis, while in zone 3 they must be treated with suspicion.

Table 3 summarises the multiple regression coefficients and R^2 for each species in the three zones. Several of the equations have been selected from two or more possible solutions. Alternative solutions will be discussed below. As already suggested, *Oithona*, *Elminius*, *Polydora* and *Coscinodiscus*

TABLE 3—Partial Regression Coefficients

Species	Zone	Temperature ($0{\cdot}1^{\circ}C$)	Salinity ($0{\cdot}1\,^{0}/_{00}$)	*Paracalanus*	*Oithona*	*Elminius*	R^2
Paracalanus	1	.....	.....	.....	0·58**	.....	0·34
	2	$1{\cdot}81^{-1}$	1·86†	.....	1·33***	.....	0·59
	3	.....	.....	.....	1·37**	.....	0·37
Oithona	1	.....	.....	0·47*	.....	0·48*	0·51
	2	1·64*	$1{\cdot}53^{-1}$***	0·36***	.....	0·29	0·95
	3	0·98***	.....	0·20**	.....	0·38**	0·75
Elminius	1	.....	.....	.....	0·49*	.....	0·29
	2	1·31	$1{\cdot}34^{-1}$	.....	0·40†	.....	0·90
	3	1·37	$1{\cdot}22^{-1}$	.....	0·73**	.....	0·39
Polydora	2	1·07*	$1{\cdot}09^{-1}$***	.....	.....	.....	0·77
Coscinodiscus	2	2·39*	$3{\cdot}02^{-1}$***	.....	.....	.....	0·92

react in similar manner to temperature and salinity, although the effect is pronounced only in zone 2. The regression is positive on temperature and negative on salinity in each case. For *Elminius,* neither coefficient is significant on its own, but in zone 2 the two combined reach significance at the ($P = 0{\cdot}05$) level. (The test for combined significance is most conveniently made by multiplying together the two probabilities as obtained by Student's t test. For temperature and salinity these are respectively 0·32 and 0·12, so the probability that *both* coefficients are estimates of zero is about 0·04). If the regression of *Elminius* on *Oithona* is eliminated from zone 2, the equation becomes:

$$\hat{N} = \bar{n}1{\cdot}85^{*t}1{\cdot}77^{***\cdot s}, \; R^2 = 0{\cdot}88$$

Both coefficients are significant in this equation. In zone 3 the T and S coefficients for *Elminius* are retained, in analogy to zone 2, although their elimination affects a negligible change in the coefficient of regression on *Oithona.* Further evidence of the reality of *Elminius*:T-S regression has been found (Cassie 1959a), where the same relationship existed and was highly significant.

Paracalanus, on the other hand, exhibits negative regression on temperature, and positive on salinity, the exact converse of the relationship for the above four species. Here again significance ($P = 0{\cdot}05$) is only reached when the two coefficients are considered together, but supporting evidence is available, the same type of regression having been reported previously (Cassie 1959a) for this species. It is noteworthy that there is no hint of this relationship in the total correlations, before partial regression on *Oithona* has been eliminated. In zone 3, *Paracalanus* has a negative regression on salinity which has been omitted from Table 3. Incorporating this regression, the equation becomes:

$$\hat{P} = \bar{p}2{\cdot}75^{***\cdot s}\, o^{0{\cdot}92^{**}}, \; R^2 = 0{\cdot}83$$

In spite of the high statistical significance of the partial regression on salinity, there are three circumstances which have led to its rejection from the table. Firstly, it is inconsistent with all other observations of *Paracalanus* which have been made in the present, and in a previous experiment (Cassie 1959a), both in its negative sign, and in the absence of an accompanying negative regression on temperature. The coupling of opposite-sign regressions on temperature and salinity would indeed seem to be a common feature of a number of plankton species, leaving this one instance as an isolated exception. Secondly, only slight changes in the salinity readings could have completely obliterated this correlation. It has already been noted that the salinity variation is only slightly greater than the limits of accuracy of the recorder. Thirdly it will be seen from Fig. 3 that regression of log. *Paracalanus* on salinity in zone 3 arises from a simple linear trend of both variates, and that deviations of one curve from this trend are not reflected by similar deviations in the other curve. In the absence of other supporting evidence, it would appear that these two trends may be attributable to independent causes and this correlation purely adventitious. It must be emphasised that "highly significant" correlations will at times occur by chance, and that

their reality must always be assessed in relation to other relevant information which may completely alter the "probability" of any suspected causal connection.

In the interspecific regressions the correlation of *Oithona* to *Elminius* and *Oithona* to *Paracalanus* is found consistently in all equations referring to these species, and is clearly independent of temperature or salinity effects. (Since the relationship is reciprocal, the term "correlation" is in this case more appropriate than "regression"). Only in one case, *Oithona:Elminius,* in zone 2, is the regression not significant, although its reality is accepted in analogy with the other zones. However, if *Elminius* is eliminated from this equation, there is no substantial change in the remaining three coefficients:

$$\hat{O} = \bar{o}\ 1{\cdot}94^{**t}\ 1{\cdot}46^{***-s}\ p^{0.28},\ R^2 = 0{\cdot}92$$

Polydora and *Coscinodiscus* do not have any clear partial regression on any of the other species, although a near-significant negative regression of *Paracalanus* on *Coscinodiscus* may be shown in zone 2:

$$\hat{P} = \bar{p}\ 1{\cdot}60^{\ t}\ 1{\cdot}47^{s}\ o^{\ 1.52***}\ c^{\ -0.34}\dagger,\ R^2 = 0{\cdot}67$$

and in zone 3:

$$\hat{P} = \bar{p}\ 0{\cdot}51^{t}\ o^{\ 1.74**}\ c^{\ -0.35},\ R^2 = 0{\cdot}59$$

Melosira alone appears to be completely independent of both physical properties and other species with the exception of a possible negative correlation to *Coscinodiscus* in zone 2 ($P = 0{\cdot}17$). From the statistical point of view, absence of correlation could be attributable in part to the different mode of sampling and extraction, or to the fact that this species is a colonial form, for which the appropriate unit to count is not altogether evident. On the whole, however, it seems likely that *Melosira* is independent of the other known variables. This observation will be considered in more detail in the discussion and conclusion.

HOMOGENEITY BETWEEN ZONES

From Table 3 it is seen that, with minor exceptions, there is consistency in sign between corresponding partial regression coefficients for the three zones. On the other hand, neither the means (adjusted for regression) the residual variances (independent of regression) nor the regression coefficients are homogeneous between the three zones. This has been verified by appropriate analyses of variance which need not be quoted. Heterogeneity is in many cases evident from an inspection of Tables 3 and 4. Variances of sample counts both before (s^2) and after ($s_R{}^2$) elimination of regression variance are given in Table 4. It is interesting to note, not only the general heterogeneity, but also the change in trend between the two sets of variances. Before regression, zone 2 has the highest variance for all species but *Paracalanus*. Elimination of regression tends to overcompensate this discrepancy so that (again with the exception of *Paracalanus*) zone 2 has the lowest variance. This would suggest interaction between temperature-

TABLE 4—Variances Before (s^2) and After (s_R^2) Regression

Species	Zone	s^2	d.f.	s_R^2	d.f.
Paracalanus	1	0·0199	19	0·0139	18
	2	0·0319	18	0·0158	15
	3	0·1394	20	0·0927	19
Oithona	1	0·0197	19	0·0108	17
	2	0·0637	18	0·0044	14
	3	0·0272	20	0·0080	17
Elminius	1	0·0165	19	0·0124	18
	2	0·0558	18	0·0065	15
	3	0·0437	20	0·0316	17
Polydora	1	0·0014	19	0·0014	19
	2	0·0056	18	0·0006	16
	3	0·0042	20	0·0042	20
Coscinodiscus	1	0·1021	19	0·1021	19
	2	0·3207	18	0·0292	16
	3	0·0559	20	0·0559	20

salinity and other pattern-inducing factors, i.e. that other factors may differ in the magnitude of their effect under different temperature-salinity gradients.

The heterogeneity between zones is in contrast to earlier results (Cassie 1959a), where it was found for *Paracalanus* that variances and regressions were common to nine distinct zones. The discrepancy is not altogether surprising since, in the present instance, the water discontinuity is of a different type, representing a transition between two bodies of water of widely different origin, as compared with minor inhomogeneities within the harbour.

Owing to heterogeneity, it is not appropriate to compute any one set of equations to describe all three zones. This does not, of course, invalidate the present equations, since an unstable regression can be accounted for in various ways. The data are almost certainly incomplete (see also next section), in that only some of the pattern-inducing variates have been sampled. Thus the regression coefficients may contain extraneous components due to other unknown effects. Interaction with other factors (known or unknown) has already been noted as a possibility. It is also not unlikely that the log-linear regression function is only an approximation to the true relationship, which may contain other curvilinear components.

THE CONTRIBUTION OF REGRESSION TO OVER-DISPERSION

A simple test for departure from randomness of the residual variance has been previously employed (1959c):

$(1 - R^2)\Sigma x^2/\bar{x}$, ($\Sigma x^2$ = sum of squares of deviations from mean),

which is distributed approximately as χ^2 with n-k degrees of freedom (n = number of samples, k = number of variates).

In the present instance this test is not available, since regressions are computed from transformed data. An approximate test is:

$$\Sigma[\ (X_j - \hat{X}_j)^2/\hat{X}_j]$$

where X_j = sample count of species X in jth sample.

$\hat{X}_j$ = predicted sample count from regression equation.

This test has been applied to those species in zone 2 where R^2 is high (*Oithona, Coscinodiscus* and *Elminius*) but in all cases $P(\chi^2) < 0{\cdot}001$. It may be assumed, then, that the regression equations account for only part (though sometimes a substantial part) of the agencies inducing overdispersion. This conclusion has been anticipated in the previous section in discussing the causes of heterogeneity between zones.

DISCUSSION OF RESULTS

The information contained in the equations is summarised in Fig. 5. The outer circle of the diagram contains the six plankton species, while in the inner circle are the two physical properties, temperature and salinity. Partial regression or correlation between any pair of the eight varieties is indicated by a solid line (positive) or a broken line (negative). The thickness of the links in Fig. 5 is roughly in proportion to the level of confidence,

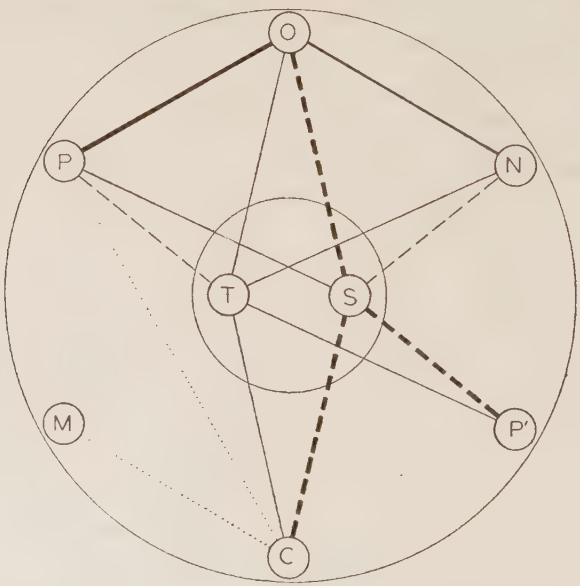

FIG. 5—Partial regression and correlation diagram. Unbroken lines: positive coefficients. Broken lines: negative coefficients. Dotted lines: doubtful negative coefficient. Thickness of connecting line approximately proportional to statistical significance level.

P, Paracalanus; *O, Oithona*; *N, Elminius* nauplii; *P', Polydora* larvae; *C, Coscinodiscus*; *M, Melosira*; *T*, temperature; *S*, salinity.

although no strict code can be employed, since there is no set of equations common to all three zones. The dotted lines between *Paracalanus* and *Coscinodiscus, Melosira,* and *Coscinodiscus* represent negative correlations of doubtful significance.

The relationships in the figure may, for the purposes of discussion, be grouped into four categories.

(1) The four species (*Oithona, Elminius, Polydora* and *Coscinodiscus*) in which regression is positive on temperature and negative on salinity.

(2) The one species (*Paracalanus*) in which regression is negative on temperature and positive on salinity (i.e. the opposite to category 1).

(3) Positive interspecific correlations (*Paracalanus: Oithona: Elminius*).

(4) Negative interspecific correlations (*Paracalanus: Coscinodiscus: Melosira.*)

(5) *Melosira,* which appears to have a pattern largely independent of the other variates.

(1) Temperature Positive, Salinity Negative

The *Elminius:T-S* relationship is of the same form previously noted (Cassie 1959a). It has been suggested that the regression is due to lower salinity and higher temperature being more favourable to the release of embryos from the parent barnacle, so that the spawning rate is controlled by the physical properties of the water circulating past them. While not necessarily invalidating this previous hypothesis, it seems more than coincidence that three other species in quite unrelated taxonomic groups should share the same relationship. Only one of the three (*Polydora*) is a larval form of a sedentary organism which might be subject to similar factors to those affecting *Elminius.*

Possibly the simplest explanation for the common relationship would be that the *Elminius* gradient in zone 2 is induced, not by temperature and salinity themselves, but by the mixing of populations from two distinct bodies of water, as represented by zones 1 and 3. However, if this were the case, since temperature and salinity are positively correlated in zone 2 ($r = 0{\cdot}97$), it would be expected that the partial regression coefficients on T and S would both have the same sign. Thus, although this hypothesis can not be entirely discounted, it is clear that either temperature or salinity of both must have some influence independent of the water mixing effect.

The opposite signs of the temperature and salinity regressions suggest that these two physical properties are acting in the role of density-determining factors. The two components would then augment each other, since a rise in salinity or a fall in the temperature both produce increased density. Density in turn may influence vertical distribution of plankton so that it will be desirable in future experiments of this type to sample vertically as well or horizontally in order to elucidate this factor. Table 5 gives total correlations with temperature, *T,* salinity *S,* and density *D,* and multiple correlations with temperature and salinity, *T-S.* (Note that the multiple correlation coefficients have no sign, so that it is appropriate to compare magnitudes but not signs with the total correlations). The high correlation of *D* and *S*

TABLE 5—Total Correlations, Log. Species to Temperature, Salinity, Density; Multiple Correlations to Temperature and Salinity

Zone		S	D	Log. P	Log. O	Log. N	Log. P′	Log. C	Log. M
1.	*T*	− 0·55*	− 0·80***	0·32	− 0·12	− 0·36	− 0·23	− 0·10	0·46*
	S		0·97***	− 0·09	0·20	0·17	0·60**	− 0·16	− 0·12
	D			− 0·18	0·13	0·19	0·63**	− 0·23	− 0·18
	T-S			0·34	0·20	0·36	0·61*	0·28	0·49
2.	*T*	0·97***	0·95***	− 0·07	− 0·75***	− 0·80***	− 0·80***	− 0·87***	− 0·20
	S		0·999***	− 0·16	− 0·86***	− 0·90***	− 0·87***	− 0·95***	0·26
	D			− 0·18	− 0·88***	− 0·91***	− 0·87***	− 0·95***	− 0·27
	T-S			0·36	0·93***	0·94***	0·88***	0·96***	0·57*
3.	*T*	0·48*	0·45**	− 0·07	− 0·40	− 0·17	0·13	0·45*	0·31
	S		0·995***	− 0·83***	− 0·28	− 0·33	0·16	0·23	0·07
	D			− 0·81***	− 0·29	− 0·34	0·16	0·21	0·08
	T-S			0·91***	0·41	0·33	0·17	0·68**	0·39

indicates that salinity alone is sufficient to account almost completely for the density changes. In most species there is little to choose between the *D* and *S* coefficients, although the *T-S* multiple correlation coefficient is usually slightly higher in value than the *D* coefficient. However, for *Polydora* (*P'*) the *D* and *T-S* coefficients are virtually identical, indicating that the temperature and salinity are contributing to the variance of this species in almost exactly the proportion which would be expected if density were the only determining factor.

A further function of temperature and salinity is viscosity which would modify rates of sinking or of locomotion. This factor, however, seems less likely to play a dominant part in the relationship, since within the existing ranges the contribution of temperature to viscosity will be about 20 times as great as that of salinity whereas in all the regression equations salinity is consistently the stronger influence.

(2) Temperature Negative, Salinity Positive

The *Paracalanus: T.-S* relationship is also in substantial agreement with previous results (Cassie 1959a). It was found that the slopes of the regressions of *Paracalanus* on temperature and salinity were consistent over nine different water zones, each separated from its neighbours by sharp (though probably relatively ephemeral) hydrological discontinuities, but that the mean population of each zone was independent of regression. A similar situation does not exist in the present experiment, since zone 2 is itself a broad discontinuity rather than a discreet body of water. Nevertheless, the relative smoothness of its gradient suggests that it is a more stable hydrological feature, and its influence on *Paracalanus* is more akin to that of the within-zone *T-S* heterogeneity than to the discontinuities between zones found previously (Cassie 1959a). The signs of both regressions are reversed as compared with the previous group of four species. This difference may be related to the fact that *Paracalanus* is commonly an oceanic species, while the other four are more typically inshore in their distribution. Once again, the opposite sign of temperature and salinity coefficients is consistent with a density effect, although it is scarcely to be expected that the *D* and *T-S* correlations will agree in Table 5, owing to the antagonism of the very much stronger regression on *Oithona* and *Elminius*.

(3) Positive Interspecific Correlations

There are strong partial correlation bonds in Fig. 5 connecting *Oithona* to both *Paracalanus* and *Elminius*. This correlation appears consistently in all three zones, and is thus the most clearly demonstrated relationship to be found in this set of data. However, there is no suggestion of any independent correlation between *Paracalanus* and *Elminius*, although the same two species were found to be correlated in a previous experiment (Cassie 1959b). An example of the relative impermanence of interspecific correlations has already been noted (1959c) and the suggestion has been made that such relationships may in some instances be due to the influence of an external physical factor, rather than to any direct influence of species on species. Barnes and Hasle (1957) make a similar observation for two species of dino-

flagellates in Oslo Fjord. These were on one occasion negatively, and eight days later positively correlated, in both cases, with significance at the ($P<0{\cdot}001$ level).

(4) NEGATIVE INTERSPECIFIC CORRELATIONS

The weak negative correlations involving *Coscinodiscus, Melosira* and *Paracalanus* can scarcely be considered as more than tentative evidence. In fact these correlations have only been discussed at all because of the relative scarcity of negative interspecific correlations both in this experiment and in previous investigations of this series, even though there seems to be no *a priori* reason for predominance of the positive sign.

(5) NO CORRELATION

Apart from the very doubtful relationship referred to above, *Melosira* appears to be completely free of any association with other species, or physical variables, a fact which might almost have been anticipated from the distinctive pattern of distribution seen in Fig. 4. This negative information may possibly be accounted for in some measure by the reproductive cycle of this species. It has been encountered at various localities in the Wellington and Cook Strait region, including Port Nicholson, Kaikoura and Cape Campbell (East Coast, South Island), and as a pronounced bloom in the Wainuiomata reservoir which overflows into the Wainuiomata river, entering the sea on the south coast about three miles S.E. of the point where sampling commenced (see Fig. 1) (U. V. Cassie, pers. comm.). Although evidence is not complete, it appears that *Melosira* may bloom rapidly and locally, spreading from small inoculation centres and declining again before time has permitted either complete dispersal or the impressing of any external pattern on its distribution.

DISCUSSION OF PATTERN-INDUCING AGENCIES

In summarising results of this experiment, consideration will also be given to those previous investigations by the author to which reference has been made freely in the text.

The pattern-forming agencies determining spatial distribution of a plankton species may be divided into four categories, attributable to:

(1) Chance,
(2) The physical environment,
(3) The influence of other species,
(4) Internal behaviour patterns within the species.

(1) CHANCE OR RANDOM FACTORS

In the absence of the other three factors it may be assumed that distribution will be random. Since most plankton organisms are small in volume as compared with their environment, the Poisson model is appropriate. The variance will approximate to the mean, and this will, for most practical purposes, represent the minimum value of the variance. Analogy may be drawn between Poisson variance and "noise level" in a communication circuit, which

represents the limit to amplification beyond which it is no longer possible to extract further information from the system. However, the analogy is not completely successful in that it is possible under certain circumstances (negative contagion) for variance to be less than the mean. This exception will be discussed under (4) below.

(2) PHYSICAL ENVIRONMENT

From temperature and salinity regressions it has been shown that certain organisms may be sensitive to relatively small variations in space of these two physical properties. The reaction is revealed by a geometric increase or decrease in the number of individuals which accompanies a linear increase in the physical variate (using "increase" and "decrease" in the spatial rather than the temporal sense). This relationship may be described by the expression:

$$Y \propto \gamma^{Z}$$

where Y is the number of individuals in a parcel of water of unit volume, Z is the mean value of the physical property in the parcel, and γ is a constant. While observations on this type of relationship have been confined to temperature and salinity, it is likely that other physical (or chemical) properties will have a similar influence on spatial distribution. Although the causation of this reaction is not yet fully understood, it has been shown that the results may at times be substantial, as witness the 300% numerical increase in *Coscinodiscus* which accompanied at 0·1% lowering of salinity in zone 2 (Table 3). Where comparable data have been obtained for the same species at different times and places, temperature and salinity regressions have proved to be consistent in sign (though not in magnitude). Temperature and salinity changes of the same sign have almost invariably had antagonistic (i.e. opposite sign) effects on abundance of any one plankton species. This suggests that both factors may be effective through their influence on density, although further information is desirable to test this assumption.

(3) OTHER SPECIES

Positive correlations between species are of frequent occurrence, and, in the present experiment, are in many cases both highly significant, and consistent in sign through different parts of the range. On the other hand, inconsistencies between different sets of samples at different times and places, suggest that the relationship between any two species is not intrinsic to that particular species combination, but may result from a common reaction to an external (possibly physical) variate in the environment. Where "predator-prey," "grazing," or "animal exclusion" relationships (Hardy & Gunther, 1935) are suspected there is no apparent reason why negative correlations should not be as common as positive. It may, then, be significant that negative correlations in this series of experiments have been few and so low in significance that they would scarcely have warranted mention had they been positive. (Note, however, the highly significant negative correlation between two dinoflagellate species which is reported by Barnes and Hasle, 1957, and has been commented on above.)

In view of the extensive literature on the inter-relationship between zooplankton and phytoplankton, it is surprising that there is so little evidence of any association between the dominant animal and plant species. Bainbridge (1953) in a series of laboratory experiments provides some relevant data. He found that various zooplankton species appeared to be attracted by *Nitzschia closterium,* a species also recorded in the present experiment. Although the present data are insufficient for regression analysis, a careful perusal of the raw data has failed to reveal any significant deviations in the zooplankton distributions which could be attributed to the presence of *Nitzschia* aggregations.

Where interspecific correlations are concerned, there is no clearly-marked preference for any one mathematical model. However, the logarithmic transformation has proved both convenient and consistent in its results, so that the relationship may be described by the expression:

$$Y \propto X^{\beta}$$

where X and Y are the numbers of two species in a unit parcel of water and β is a constant. Note, however, that the role of dependent variate is interchangeable between X and Y since there are no grounds for postulating that either species is controlled by the other. It has, in fact, already been shown in the "Statistical Methods" section that the log. interspecific correlation is consistent with a common regression of the two species concerned on a physical variate.

(4) Internal Behaviour Patterns Within the Species

Non-random spatial patterns which are entirely independent of external influences may conveniently be classified as contagious distributions. While the terms "positive and negative contagion" have sometimes been employed to describe all types of over and underdispersion, the restricted use suggested above would seem a useful distinction between two sets of terms which are otherwise synonymous.

Underdispersion (variance<mean) is comparatively rarely observed, although it has been reported by Ricker (1937) and others. This apparent rarity is possibly due to problems of detection since any external factor tending to increase the variance will automatically obscure the underdispersion pattern. It would seem reasonable to interpret underdispersion when it does occur as an avoidance reaction between individuals of the same species, i.e. negative contagion, since external conditions could probably induce this condition only rarely. Alternatively, negative contagion could be considered as a "non-Poisson random distribution." If each organism is regarded as having a volume which includes not only its body, but also a certain "territory" the individual might no longer be of negligible size in relation to its environment. Distribution would then be binomial with variance, $\sigma^2 = mq$ where m is the mean number of planktons per sample, q is the proportion of the total habitat not filled by plankton "territory." The Poisson variance, $\sigma^2 = m$ is the limiting case where q approaches unity. If, however, q is less than unity, the variance will be less than the mean.

Positive contagion will also, in practice be somewhat difficult to detect, since it can only be demonstrated in an environment which is entirely homo-

geneous or in which all the parameters of heteorogeneity are known. Mechanisms likely to induce positive contagion may be divided into two general categories. Firstly the initial disposition (either from immigration or reproduction) of a group of organisms when it first colonises a region will not become Poisson until sufficient time has elapsed for complete dispersal.* Secondly, individuals may tend to attract each other (the converse of the avoidance reaction inducing negative contagion).

An example of the first category is given by the teleost eggs discussed by Cassie (1959b). These eggs were only a few hours old and were arrayed in a simple diffusion pattern about a centre where they were presumably deposited by the parent fish. *Melosira* and *Nitzschia* in the present experiment probably owe their distribution to similar causes since their pattern is consistent with a series of intense blooms, initiated perhaps by external factors, but arising from a few scattered and previously dormant cells.

For the second category of positive contagion there is as yet no definite information. Autocorrelation between numbers of individuals in adjacent samples would at first appear to be presumptive evidence for a mutual attraction between individuals. However, where such a relationship has been detected in previous experiments (1959a,) other interpretations have almost invariably been possible. Even when autoregression has remained significant after partial analysis with other factors, the information gained (as judged by the increase in R^2) has been negligible.

The lack of positive evidence does not of course disprove the existence of gregarious contagion. Nevertheless it does indicate the need for caution in ascribing a behaviour pattern to a species when it may in fact be a function of the environment.

CONCLUSION

The above classification of pattern-forming agencies into four categories, while forming a useful working model, is undoubtedly an oversimplification. There is no absolute dividing line between elemental properties of the environment (such as temperature and salinity) and those properties which are attributable in part to the presence of other organisms of the same or other species (such as pH, dissolved oxygen). Similarly interspecific and intraspecific behaviour are not necessarily independent phenomena. The "gregarious" behaviour of one species may be a means of ensuring economy of sexual products in spawning, a defensive reaction to the predations of a larger species, or a cooperative measure in preying on a smaller species.

Even more fundamental than the lack of exclusive categories is the possibility of interaction between different factors, so that the combined effect of changing two or more factors is greater (or less) than the simple

*Note that the terms *dispersal* and *dispersion* are not synonymous in the present context. *Dispersal* refers to the normal ecological concept of individuals migrating outwards from their point of origin to fill a given habitat. *Dispersion* is a statistical term referring to the spread of individual observations (e.g. sample counts) about the mean. Unfortunately the distinction is not always observed in the ecological literature. This often causes confusion since increased *dispersal* of individuals leads to decreased *dispersion* of sample counts.

algebraic sum of individual effects. The lower residual variance of zone 2 after temperature-salinity regression has been eliminated is possibly an instance of interaction.

In practice the minimum error or "noise level" will usually be somewhat greater than Poisson even when counts and measurements are without error or bias. This additional error component will arise from inability to make the sampling pattern fine enough to account for the smaller spatial elements of the population pattern. The impracticability of obtaining these ultimate details has been demonstrated in a previous case where overdispersion was still detectable in a line of samples at 10 cm intervals (Cassie 1959b).

While the foregoing discussion is based on incomplete evidence, it would seem that the non-random spatial pattern of plankton is capable of explanation in terms of relatively simple causal factors, complexity arising only from the mulitplicity of these factors. Even if an inventory of the entire complex is beyond present resources it should be possible in many instances to make a substantial reduction in the uncertainty of ecological plankton studies (or indeed of any ecological studies) by extending the concepts outlined in this discussion.

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STUDIES IN POLLEN MORPHOLOGY

1. THE NEW ZEALAND SPECIES OF *HYDROCOTYLE* LINN.

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Summary

The pollen morphology of the eight indigenous species of *Hydrocotyle* is described and four groups, based on exine characters and size range, are recognised. Pollen grains of a large-leaved *Hydrocotyle* from the Chatham Islands are also described. The relationship of *Hydrocotyle* to other genera of the Umbelliferae and to the Araliaceae is briefly discussed. Three species, *Hydrocotyle microphylla, H. novae-zelandiae* and *H. tripartita,* have been identified in recent pollen analyses.

Introduction

Hydrocotyle, Subfamily Hydrocotyloideae, of the family *Umbelliferae* is a large genus of about 75 species, the main areas of distribution being in the Southern Hemisphere. Of the eight species in New Zealand five are endemic, one occurs in North and South America and two are found in Australia (Cheeseman, 1925). Although no published papers discuss in detail the pollen morphology of the genus there are available brief descriptions of some species (Erdtman, 1924, 1943, 1952; Fischer, 1890; Hafsten, 1951; Ikuse, 1956; Selling, 1947).

Since umbelliferous pollen grains occur frequently, but not in large numbers, in most peat profiles examined in New Zealand, detailed descriptions of these should assist in their identification, and assist in the interpretation of the flora at the site of deposition.

The descriptions and measurements are based on acetolysed material (Erdtman, 1952).

Pollen Morphology

Exine: The exine is very thin (about 1μ) but is always thicker at the poles, especially in the species *Hydrocotyle novae-zelandiae, H. pterocarpa,* and *H. tripartita,* where it may be as thick as 2–3μ. The thicker polar exine is usually slightly offset from the thinner areas of the exine, a feature which is particularly obvious in *H. elongata, H. novae-zelandiae,* and *H. pterocarpa* (Fig. 1), and which has been described for *H. verticillata* by Selling (1947). This character does not occur in *H. novae-zelandiae* var. *robusta* nor in a large-leaved, undescribed species (Cheeseman, 1925) from the Chatham Islands. In these, the exine gradually thickens towards the poles (Fig. 2).

The exine is too thin to enable discernment of any structural characters except at the poles of those pollen grains with the thickest exine where bacula are clearly visible (Fig. 1). The outer ends of these bacula are fused so that in the polar area the exine is clearly tectate (Hafsten, 1951; Selling, 1947). The exine surface is not strongly sculptured, and in some species e.g. *Hydrocotyle moschata*, it is smooth or faintly flecked. A coarser sculpturing, apparently a fine reticulum, serves to separate *H. novae-zelandiae*, *H. pterocarpa*, and *H. tripartita* from the smooth-walled species.

APERTURES: The pollen grains are typically tricolporate although in some species tetra- and hexa-colporate grains do occur.The colpae are long, reaching nearly to the poles, and narrow, and the equatorial pores are rectangular and lie at right angles across the furrow, i.e. transversal furrows (Faegri and Iversen 1950). Pollen with such apertures are common in the Umbelliferae and occur also in the Araliaceae e.g. in *Nothopanax*. The ektexine is bulging, or pouting, at the pores, particularly so in *Hydrocotyle americana*, *H. dissecta*, and *H. moschata*, a feature also of *H. verticillata* and *H. vulgaris* (Erdtman 1924, 1943, 1952; Selling, 1947).

Tetracolporate pollen grains (Figs. 1g, 2a) occur in *H. americana*, *H. dissecta*, *H. elongata*, *H. moschata*, and in *H. novae-zelandiae* var. *robusta*. On one slide of *H. americana* (CHR 65903, Sl 4081) 29% of the pollen counted was tetracolporate, and on one slide of *H. novae-zelandiae* var. *robusta* (WELT 10837, Sl 4279) 62% of the pollen counted was tetracolporate; of the slides of other species less than 1% was tetracolporate. In addition a few hexacolporate grains were seen on most slides of *H. moschata*. The author has been unable to find reference to such pollen in the literature.

SHAPE: The pollen grains, elongated along their polar axes, are sub-prolate with the exceptions of the long and narrow *Hydrocotyle novae-zelandiae*, which is prolate, and *H. tripartita*, the pollen of which is rather variable and may be either prolate or sub-prolate. *H. americana* and *H. pterocarpa* occasionally have prolate-spheroidal pollen grains.

In equatorial view the poles are seen to be sharply angled, and are less rounded than most other Umbelliferae pollen grains. In some species, particularly *Hydrocotyle moschata*, and an undescribed species from the Chatham Islands, the pollen grains are also sharply angled at the equator (Fig. 2b).

In polar view the pollen of all indigenous species are angulaperturate, the equatorial limb being straight as in *H. moschata*, or convex as in *H. novae-zelandiae*, so that the pollen may be triangular, sub-triangular, or sometimes, nearly spheroidal.

In her pollen key Cranwell (1942) stated that many of the Umbelliferae are dumb-bell shaped when unexpanded, but this is not a character of the New Zealand species of *Hydrocotyle*, nor of *H. verticillata* (Selling, 1947). Erdtman (1952) has stated that there are no grains with equatorial constrictions in the Hydrocotyloideae.

SIZE: Pollen grains were measured along the equatorial and the polar axes, and whenever possible fifty grains on any one slide were measured. On the basis of size measurements the New Zealand species fall into two groups and these can be compared with the few records of species not occurring in this country (Table 1).

TABLE 1—Size Range in *Hydrocotyle* Species

Country of Origin	Species	Size Range (μ) Polar Axis	Equatorial Axis	Source
New Zealand	*Hydrocotyle dissecta*	20(22)25	15(18)22	.....
	H. microphylla	20(22)25	15(16)17	.....
	H. moschata	20(23)27	17(18)20	.....
		20(26)30	17(21)25	.....
	H. americana	20(23)25	20(21)22	.....
	H. elongata	22(24)27	15(18)20	.....
	H. pterocarpa	22(24)27	17(21)25	.....
	H. tripartita	27(29)32	20(22)25	.....
	H. sp. (Chatham Islands)	30(32)35	20(24)25	.....
	H. novae-zelandiae var. *robusta*	30(33)37	22(27)30	.....
	H. novae-zelandiae	30(34)37	20(22)25	.....
Europe	*H. vulgaris*	21 23	17 20·5	Erdtman (1943) Erdtman (1952)
Hawaiian Islands	*H. verticillata*	28	20	Selling (1947)
Japan	*H. maritima*	19·5–21	15–17	Ikuse (1956)
Tristan da Cunha	*H. capitata*	28	22	Hafsten (1951)

The two size classes recorded for *Hydrocotyle moschata* are apparently unusual in the genus, but there are records of two size classes in other groups of the Umbelliferae (Selling 1947).

DESCRIPTIONS OF POLLEN

Hydrocotyle moschata Forst. f. Fig. 1g, k.

Free, radiosymmetrical, isopolar; typically tricolporate, some tetracolporate grains present; furrows long, narrow, reaching almost to poles, margins slightly thickened; transversal furrows rectangular, up to 5μ long; exine thin, raised or pouting at pores, slightly thicker at poles and slightly offset; surface smooth; sub-prolate P:E :: 8:6, angulaperturate; size variable, polar axis: 20(23)27μ, 22(26)30μ; equatorial axis: 17(18)20μ, 17(21)25μ.

Tetracolporate grains occur in low frequencies, and have shorter furrows, tend to be oblate, and are about the size of the larger group of grains (Fig. 1k).

MATERIAL: CHR 93156, Sl 4070*, Wadestown, Wellington; CHR 73631, Sl 4071, Palliser Bay; *J. B. Hair 348,* Sl 4113, Mt. Blowhard, North Can-

*CHR and number refers to the sheet number of a specimen in the Botany Division Herbarium, D.S.I.R. WELT and number refers to the sheet number of a specimen in the Herbarium of the Dominion Museum. Sl and number refers to the slide number in the Divisional Slide Collection.

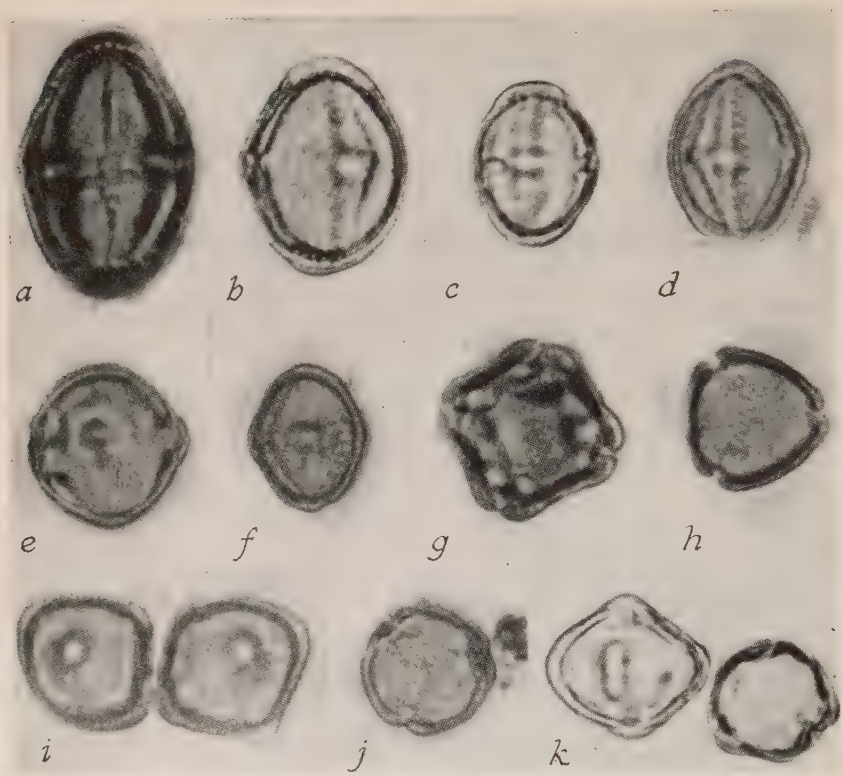

FIG. 1—Pollen grains of indigenous species of *Hydrocotyle* in optical section. Equatorial views, a-f, i, k. Polar views, g, h, j, k.
(a) *Hydrocotyle novae-zelandiae,* (b) *H. tripartita,* (c) *H. pterocarpa,* (d) *H. elongata,* (e) *H. americana,* (f) *H. microphylla,* (g, k) *H. moschata,* (h, i) *H. dissecta,* (j) *H. pterocarpa.* Magnification × 1,000.

terbury; Sl 4126, Botany Division lawn, Christchurch; Sl 4124, Mt Herbert, Banks Peninsula; CHR 94141, Sl 4064, Port Levy Road, Banks Peninsula; CHR 78917, Sl 4067, Preservation Inlet, Stewart Island.

Hydrocotyle americana Linn. Fig. 1e.

Free, radiosymmetrical, isopolar; typically tricolporate, but some tetracolporate; furrows long, narrow, extending almost to poles, margins slightly thickened; transversal furrows rectangular; exine very thin, slightly pouting at pores; slightly thicker at poles and slightly offset; surface faintly flecked; sub-prolate, to prolate-spheroidal, P:E :: 8:7, in polar view angulaperturate, sub-triangular; polar axis: 20(23)25μ, equatorial axis: 20(21)22μ. Tetracolporate grains have shorter furrows, are rhomboidal and angulaperturate in polar view, oblate spheroidal in equatorial view (P:E :: 7:8) and are slightly longer than tricolporate grains.

Material of variety *heteromeria* was not available.

MATERIAL: CHR 68503, Sl 4080, Pohangina; CHR 65903, Sl 4081, Waikanae.

Hydrocotyle dissecta Hook. f. Fig. 1h, i.

Free, radiosymmetrical, isopolar; tricolporate and tetracolporate, occasionally hexacolporate. Tricolporate grains: furrows long, narrow, reaching almost to the poles, margins slightly thickened; transversal furrows rectangular; exine very thin, pouting at pores, thicker at poles; surface smooth or faintly flecked; sub-prolate P:E :: 8:6·5; polar view angulaperturate; subtriangular; polar axis: 20(22)25μ, equatorial axis 15(18)22μ. Tetracolporate grains: furrows shorter, rhomboidal, angulaperturate in polar view.

MATERIAL: CHR 11238, Sl 4065, Korere, Nelson; CHR 23479, Sl 4066, between Nydia Bay and Tennyson Inlet, Queen Charlotte Sound.

Hydrocotyle microphylla A. Cunn. Fig. 1f.

Free, radiosymmetrical, isopolar; tricolporate, furrows long, narrow, tapering, reaching almost to poles; transversal furrows rectangular; exine very thin, pouting at pores, thicker and slightly offset at the poles; surface faintly flecked; subprolate P:E :: 8:6; polar view angulaperturate, subtriangular; polar axis: 20(22)25μ, equatorial axis: 15(16)17μ.

MATERIAL: CHR 51590, Sl 4075, N.W. Nelson.
SUB-FOSSIL: A few grains have been recorded from peats in the Ruahine Ranges.

Hydrocotyle elongata A. Cunn. Fig. 1d.

Free, radiosymmetrical, isopolar; tricolporate; furrows long, narrow, tapering, reaching almost to poles, margins raised; transversal furrows rectangular, sometimes almost ellipsoidal; exine very thin, pouting at pores, thickened and slightly offset at poles, tectate, rods faintly visible; surface flecked or faintly reticulate; sub-prolate, P:E :: 8:6; polar view angulaperturate, subtriangular; polar axis: 22(24)27μ, equatorial axis: 15(18)20μ. An occasional tetracolporate grain was noted.

MATERIAL: CHR 528, Sl 4072, Bunnythorpe; CHR 81575, Sl 4069, Westport; *J. B. Hair, 668,* Sl 4114, Sl 4155, Peel Forest, Canterbury.

Hydrocotyle pterocarpa F. Muell. Fig 1c.

Free, radiosymmetric, isopolar; tricolporate, furrows long, narrow, slightly tapering, reaching almost to poles; transversal furrows rectangular, up to 7μ long; exine thin, thicker (2μ) at poles, noticeably offset from thinner exinous areas, tectate, bacula obvious; surface finely reticulate; subprolate occasionally prolate spheroidal, P:E :: 8:7; polar view sub-triangular to spheroidal; polar axis: 22(24)27μ; equatorial axis: 17(21)25μ.

MATERIAL: CHR 65641, Sl 4074, Waikanae.

Hydrocotyle tripartita R. Br. ex A. Rich. var. *tripartita.* Fig. 1b.

Free, radiosymmetric, isopolar; tricolporate; furrows long, narrow, tapering, reaching almost to poles; transversal furrows not conspicuous; exine very

thin; thicker at poles (2·5μ) and offset, tectate, bacula obvious at poles; surface finely reticulate, coarser at poles; prolate, P:E :: 8:6; polar view, angulaperturate, sub-triangular; polar axis: 27(29)32μ, equatorial axis: 20(21)25μ.

MATERIAL: CHR 107153, Sl 4129, Westland; CHR 11217, Sl 4076, no locality.

H. tripartita var. *hydrophila* (Petrie) Cheesem.

Available material was inadequate for detailed study, but it would appear that pollen of this taxon is similar to that of the typical variety.

MATERIAL: CHR 90954, Sl 4077, Pauri Lake, Wanganui.
SUB-FOSSIL: A few grains have been recorded from peats in the Ruahine Ranges.

Hydrocotyle novae-zelandiae DC var. *novae-zelandiae*. Fig. 1a.

Free, radiosymmetric, isopolar; tricolporate; furrows long, narrow, tapering, reaching almost to poles, margins slightly thickened; transversal furrows large, rectangular, up to 10μ long; exine thin, thicker (2·5μ) and obviously offset at poles, tectate, bacula obvious at poles; surface finely reticulate; prolate, P:E :: 8:5; polar view, angulaperturate, sub-triangular to spheroidal; polar axis: 30(34)37μ, equatorial axis: 20(22)25μ.

MATERIAL: CHR 10067, Sl 4134, Arthur's Pass; CHR 93632, Sl 4068, Five Finger Peninsula, Resolution Island.

SUB-FOSSIL: One grain has been found in a buried peat at Ilam, Christchurch.

H. novae-zelandiae DC var. *robusta* Cheesem. Fig. 2a.

Free, radiosymmetric, isopolar; tricolporate, but mainly tetracolporate, rarely hexacolporate; furrows long, reaching almost to poles; transversal furrows, short, often rounded; exine thin in equatorial areas, gradually thickening towards the poles (up to 2μ thick), not obviously offset; tectate, baculate, rods visible, especially at poles, surface finely reticulate; exine at pores slightly "pouting"; prolate to sub-prolate (P:E :: 8:7); polar view angulaperturate, sub-triangular to spheroidal; polar axis: 30(33)37μ, equatorial axis: 22(27)30μ.

MATERIAL: WELT 10837, Sl 4279, Port Tryphena, Gt. Barrier Island; WELT 10869, Sl 4284, Gt. Barrier Island.

H. novae-zelandiae DC var. *montana* T. Kirk

Pollen grains are identical with the typical variety.

MATERIAL: *J. B. Hair 350*, Sl 4291, Mt Blowhard, North Canterbury; WELT 10867, Sl 4286, Fortrose, sandy spots by sea; WELT 10863, Sl 4280, Clarence Valley, Inland Kaikouras; WELT 10864, Sl 4283, Lake Wakatipu.

Hydrocotyle sp. Fig. 2b

Free, radiosymmetric, isopolar; tricolporate; furrows long, reaching almost to poles, transversal furrows rectangular, slightly constricted in middle; exine

thin in equatorial areas, slightly thicker and "pouting" at pores, gradually increasing in thickness towards the poles where it is up to 2μ thick; tectate, bacula clearly visible in polar exine; surface coarsely flecked, prolate to subprolate (P:E :: 8:6), sharply angled in equatorial view; polar view angulaperturate, sub-triangular to spheriodal; polar axis 30(32)35μ; equatorial axis 20(23)25μ.

MATERIAL: WELT 10871, SI 4282, wet lowland forest, Chatham Islands; WELT 10868, SI 4281, Chatham Islands. This latter is labelled *H. novae-zelandiae* var. *robusta*, but its pollen grains are identical with those of the first specimen cited and cannot be confused with variety *robusta*.

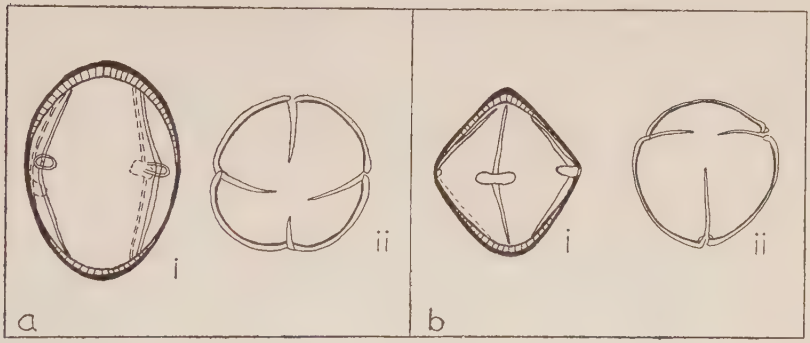

FIG. 2—a. *Hydrocotyle novae-zelandiae* var. *robusta* (i) equatorial view. (ii) polar view.

b. Chatham Islands *Hydrocotyle*. (1) equatorial view. (ii) polar view. Magnification × 1,000.

DISCUSSION

On the basis of pollen morphology the indigenous species of *Hydrocotyle* may be separated into four groups (Table 2).

High aperture number is one criterion for detecting polyploidy in groups which are usually 3-aperturate (Funke 1956), but except in the one slide of *Hydrocotyle americana* and in *H. novae-zelandiae* var. *robusta*, the numbers of tetracolporate and hexacolporate grains are so low that they may be attributed to normal variation, for it is not unusual to find some differences in aperture number when examining many pollen grains of any one species. A variable number of apertures is usual in some genera such as *Nothofagus*.

The marked differences in size range of *H. moschata* may indicate polyploidy and in this respect it is noted that the plants from which pollen of the smaller size were taken are generally more compact in habit and have smaller leaves than those plants from which the larger pollen grains were taken. Carse (1929) describes a densely matted plant with smaller leaves, fruits, and flowers, as variety *parvifolia*, which is apparently smaller than any of the plants seen in this investigation.

TABLE 2—Pollen Groups of the Indigenous Species of *Hydrocotyle*

Group	Species	Exine	Apertures	Average Size (μ)
I	*Hydrocotyle moschata* *H. dissecta* *H. americana* *H. microphylla*	Very thin and smooth. Polar exine slightly offset; bacula not visible.	Typically 3-aperturate. 4- and 6-aperturate grains present. Pores "pouting" in optical section.	23
II	*H. elongata* *H. pterocarpa*	Thin and smooth. Polar exine offset, bacula visible.	Typically 3-aperturate, 4-aperturate grains noted in *H. elongata*. Pores scarcely "pouting".	24
III	*H. tripartita* *H. novae-zelandiae*	Thin, finely reticulate. Polar exine thick, obviously offset, bacula clearly visible.	Typically 3-aperturate. Pores not "pouting".	*H. tripartita* 29 *H. novae-zelandiae* 34
IV	*H. novae-zelandiae* var. *robusta* *H.* sp. (Chatham Islands).	Thin, finely reticulate. Polar exine, thick, not offset. Bacula clearly visible.	3- and 4- aperturate.	*H. novae-zelandiae* var. *robusta* 33. Chatham Island plant, 32

Small-leaved plants growing in the Botany Division lawn are anomalous in that pollen from these is in the larger range, and as their habit may be related to the close cutting of the sward, specimens are now being grown in Botany Division Gardens for further investigation. E. J. Beuzenberg (pers. comm.) reports that the indigenous species of *Hydrocotyle* form a polyploid series, and that the differences discussed in this paper are in keeping with his recognition of a high level of ploidy in *Hydrocotyle novae-zelandiae* and *H. tripartita* var. *hydrophila*.

Kirk (1899) proposed *Hydrocotyle robusta* as a species, but with some hesitation, since "it is not easy to find good specific characters" to separate it from *H. novae-zelandiae*, and later, Cheeseman (1925) reduced it to varietal status on the grounds that the differences are of a trivial nature. The differences in pollen morphology, however, are not trivial, and the pollen grains of *H. novae-zelandiae* var. *robusta* can readily be distinguished from those of the typical variety and variety *montana* (Fig. 1a, Fig. 2a).

Cheeseman (1925) refers to a "large-leaved species gathered by Dr Cockayne in forests in the Chatham Islands," closely allied to *H. novae-zelandiae* var. *robusta*. The pollen grains of this plant are distinct from those of *H. novae-zelandiae* var. *robusta*, although having characters in common, and cannot be confused with the pollens of other indigenous species (Fig. 2b).

Hylander (1945) set up the family Hydrocotylaceae for the sub-family Hydrocotyloideae on the grounds that its characters are at least as distinctive as are the characters which separate the Umbelliferae from the Araliaceae. On the basis of pollen morphology Selling (1947) supports this view and considers that the *Hydrotcotyle* pollens known to him have characters more in common with the Araliaceae than with the umbelliferous pollens he described from the Hawaiian Islands.

The pollen of the species described in this paper have affinities both with the Umbelliferae and with the Araliaceae. Thus, the sub-prolate or prolate pollen grains of most *Hydrocotyle* species are typical of Umbelliferae, but the prolate-spheroidal grains of *H. americana* and *H. dissecta* are closer to those of some genera in the Araliaceae. The more sharply angled polar exine is typical of many Araliaceae, but this character is not a constant one, for in some araliads, e.g. the pollen of *Stilbocarpa polaris*, the poles are as rounded as are many Umbelliferae pollen grains. Many Umbelliferae pollens are constricted at the equator which is not the case with those of *Hydrocotyle*. Transversal furrows are common to both families.

Illustrations in Auer (1933) and in Auer *et al.* (1955) indicate that the pollen of at least some other genera in the Hydrocotyloideae are similar to those of the indigenous species of *Hydrocotyle*. On the other hand, the pollen of the closely related *Centella uniflora* are more like the grains of other members of the Umbelliferae. Because there is some variation within both families, and since present knowledge of the Hydrocotyloideae is limited, agreement with Hylander (1945) should be deferred until the group has been more intensively studied. It is of some interest to note that in two recent works (Hiroe and Constance, 1958; Hutchinson, 1959) *Hydrocotyle* is retained in Umbelliferae without comment, although Clapham *et al.* (1952) adopt Hydrocotylaceae in their work.

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PECTOLYTIC ACTIVITY IN XANTHOMONAS

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Summary

A study has been made of the pectolytic activities of 145 cultures representing 38 species of *Xanthomonas*. The organisms were tested for their ability to liquefy a pectin gel and to produce pectin methylesterase, pectin polygalacturonase, and protopectinase. Many organisms were found to possess enzymes that they have not previously been reported to produce but it was considered impracticable to use the presence or absence of these enzymes to identify the various species.

Introduction

The nature of diseases caused by *Xanthomonas* is not suggestive of much pectolytic activity and it is possibly for this reason that little work on this subject has been reported. Shishelova (1951) found that *Xanthomonas campestris* produced protopectinase in excised plant tissue and in living plants while *X. phaseoli* and *X. translucens* produced it only in growing plants. Sabet and Dowson (1951) reported that *X. campestris* and *X. citri* caused moderate liquefaction of a pectate gel while *X. begoniae* and *X. malvacearum* caused slight liquefaction. Smith (1958a) showed that *X. campestris* produced both pectin glycosidase (polygalacturonase) (PG) and pectin methylesterase (PME) while *X. begoniae, X. juglandis, X. malvacearum, X. phaseoli* and *X. pruni* showed neither PG nor PME. *X. vasculorum* was found to be variable since three strains gave no activity while one strain produced both PG and PME. By using a chromatographic method Smith (1958b) found that this latter strain did not liberate the lower uronides, whereas *X. campestris* liberated both mono and higher uronides. If pectin depolymerase (DP) differs from PG in that it does not liberate monogalacturonic acid (Srivastava *et al.*, 1959), it would appear (from Smith's (1958) work) that at least some strains of *X. vasculorum* produce DP.

This paper describes experiments which were carried out to determine and compare the pectolytic activities in *Xanthomonas* chiefly with a view to finding distinctions between species in the genus. At present unequivocal identification of *Xanthomonas* species by laboratory procedures is not possible.

Cultures Tested

Cultures were received from: (1) National Collection of Plant Pathogenic Bacteria, Harpenden, England; (2) Indian Type Culture Collection, New Delhi, India; (3) Department of Scientific and Industrial Research, Auckland, New Zealand; (4) College of Agriculture, Edinburgh, Scotland; (5) Dr W. A. F. Hagborg, Canada; (6) Dr E. Hellmers, Denmark; (7) Professor T. M. B. Payne, Canada; (8) Dr A. F. G. Slootweg, Holland; (9)

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Professor M. P. Starr, U.S.A. The following list gives the cultures (with name as received), the strains tested, and the code letters used: *X. abilineans* (Ashby, 1929) Dowson, 1943, ZA1, ZA3, ZA5; *X. alfalfae* (Riker *et al.*, 1935) Dowson, 1943, ZU1; *X. begoniae* (Takimoto, 1934) Dowson, 1939, Z1, Z2; *X. beticola* (Smith *et al.*, 1911) Savulescu, 1947, ZV3; *X. betlicola* Patel *et al.*, 1951, YC1; *X. campestris* (Pammel, 1895) Dowson, 1939, Q1, Q2, Q5–Q7, Q9, Q11–Q19; *X. campestris* var. *armoraciae* (McCulloch, 1929) Starr and Burkholder, 1942, Q10; *X. carotae* (Kendrick, 1934) Dowson, 1939, ZC1–ZC4; *X. cassava* Wiehe and Dowson, 1953, ZD1; *X. cassiae* Kulkarni *et al.*, 1951, YD1; *X. citri* (Hasse, 1915) Dowson, 1939, R1–R6, R8, R9; *X. cucurbitae* (Bryan, 1926) Dowson, 1939, ZE1, ZE2; *X. desmodii* Uppal and Patel, 1949, YE1; *X. geranii* (Burkholder, 1937) Dowson, 1939, YB1, YB2; *X. hyacinthi* (Wakker, 1883) Dowson, 1939, Y2, Y5, Y6; *X. juglandis* (Pierce, 1901) Dowson, 1939, S1–S15; *X. lactucae-scariolae* (Thornberry and Anderson, 1937) Savulescu, 1947, ZF1; *X. malvacearum* (Smith, 1901) Dowson, 1939 ZG1–ZG7; *X. manihotis* (Arthaud-Berthet, 1912) Starr, 1946, ZH1; *X. nakatae* (Okabe, 1933) Dowson, 1943, YF1, YF2; *X. nigromaculans* f. sp. *zinniae* Hopkins and Dowson, 1949, ZT1; *X papavericola* (Bryan and McWhorter, 1930) Dowson, 1939, ZI1–ZI3; *X pelargonii* (Brown, 1923) Starr and Burkholder, 1942, ZJ1, ZJ2; *X phaseoli* (Smith, 1897) Dowson, 1939, ZK1–ZK3; *X. phaseoli* var. *fuscans* (Burkholder, 1930) Starr and Burkholder, 1942, ZK4, ZK5; *X poinsettiaecola* Patel *et al.*, 1951, ZX1; *X pruni* (Smith, 1903) Dowson, 1939, T1, T2, T6–T13; *X. punicae* Hingorani and Singh, YG1, YG2; *X. ricinicola* (Elliott, 1930) Dowson, 1939, ZM1–ZM3; *X. rubrilineans* (Lee *et al.*, 1925) Starr and Burkholder, 1942, ZN1, ZN2; *X. sesbaniae* Patel *et al.*, 1952, YH1; *X. stewartii* (Smith, 1914) Dowson, 1939, ZO1–ZO4; *X. translucens* (Jones *et al.*, 1917) Dowson, 1939, ZQ1–ZQ13; *X. trichodesmae* Patel *et al.*, 1952, YI1; *X. trifolii* (Huss, 1907) James, 1955, ZP1; *X. uppalii* Patel, 1948, ZR1–ZR3; *X. vasculorum* (Cobb, 1893) Dowson, 1939, ZS1–ZS8; *X vesicatoria* (Doidge, 1920) Dowson, 1939, X1, X3–X5, X7, X8; *X. vignicola* Burkholder, 1944, ZY1–ZY3; *X. vitians* (Brown, 1918) Dowson, 1943, YA1, YA2. For comparative purposes two cultures of *Erwinia carotovora* (Jones, 1901) Holland, 1920, G13, G14, were also examined. Although the author considers that there is some synonymy among the species listed above and also that certain cultures should not be included in the genus *Xanthomonas*, the taxonomy of the organisms will not be discussed in this paper.

METHODS AND MEDIA

1. LIQUEFACTION: A double layer sodium pectate gel (Wieringa, 1949) was used, consisting of (a) calcium agar, i.e. K_2HPO_4,1g; $(NH_4)_2SO_4$, 1g; Ca Cl_2. $6H_2O$, 5g; agar, 15g; water 1l. pH 5·0, as the bottom layer and (b) pectate solution, i.e. A.S.P. sodium pectate (supplied by the A.S.P. Chemical Co., Gerrards Cross, Bucks., England) 20g; water 1l, pH 9·4, as the upper layer. The pectate solution was sterilised by heating to 15 lb pressure momentarily. The pectate formed a gel in approximately 30 min. The plates were dried at 45°, spot inoculated with full loops of culture and incubated at 27° for up to 5 days.

2. PECTIN METHYLESTERASE (PME): I. Using a high methoxyl pectin the growth medium was prepared as described by Smith (1958a), i.e. Solution A: Brown Ribbon pectin (a pharmaceutical grade having a methoxyl content of 12·5% on weight of pectin and obtained from Union Crystalex Gelatine Ltd., Garret St., Golden Lane, London) 5g; glucose 0·5g; water 500 ml, pH 6·6, sterilised at 15 lb pressure momentarily. Solution B: Tryptone, 2·5g; yeast extract, 1·25g; $(NH_4)_2HPO_4$, 1·25g; Na_2HPO_4, 0·025g; K_2HPO_4, 0·025g; L-asparagine monohydrate, 0·5g; water 1l, pH 6·6, tubed in 10 ml amounts. After sterilisation 2·5 ml of solution A were added to each 10 ml of solution B, then cultures were inoculated and incubated at 27° on a shaker.

II. Test solution: Brown Ribbon pectin, 0·5g; phenol, 0·2g; NaCl, 0·58g; brom thymol blue, 0·7 ml of a 1·6% alcoholic solution; water 1l. Immediately before use, the solution was adjusted to pH 6·6–6·8 with dilute NaOH. Instead of centrifuging the cultures as Smith did, 0·6 ml of culture in the growth medium was removed by sterile pipette to sterile test tubes; 7·4 ml of test solution were then added, the pH adjusted to 7·4–7·6 with NaOH and the tubes were incubated in a waterbath at 30°. Tests were made after incubation of the cultures in the growth medium for 1, 2, 3, 4, 6, 8, 10, 14, and 24 days and the results were recorded after incubation in the test solution for 2 days. A change in pH to 6·4 or below was considered to be due to the action of PME. Strong reactions were obvious within 24 hr.

3. PECTIN POLYGALACTURONASE (PG): Polygalacturonase has usually been determined (Kertesz, 1951) either by estimation of the reducing groups liberated by its action on pectic acid or by its reduction of the viscosity of the same substrate. These methods are inconvenient for use with a large number of bacterial cultures. A cup-plate diffusion technique was used with microfungi by Dingle, Reid, and Solomons (1953), who showed that it provided a quantitative estimation of the enzymes, the relationship between the diameter of the zone of diffusion and the logarithm of the amount of enzyme being linear, over a wide range. The zones of diffusion were shown by flooding the plates with reagents which precipitated products of pectin degradation.

Three media modified from that used by Dingle *et al.* (1953) were prepared as follows:

I. Sodium pectate (ASP), 10g; N.Z. (Davis) agar, 10g; yeast extract, 5g; KH_2PO_4, 1g; water 1l, pH 5·5, plus 50 ml. McIlvaine's buffer (Clark, 1928) pH 5·5. After sterilisation at 15 lb pressure momentarily, the pH was adjusted to 7·0.

II. Rotband pectin (supplied by Unipektin, Zurich, pH 4·5, and having a methoxyl content of approximately 4·5%), 10g; N.Z. (Davis) agar, 10g; yeast extract, 5g; K_2HPO_4, 1g; water 1l pH 7·0, plus 50 ml McIlvaine's buffer, pH 7·0 (pH after autoclaving 6·9).

III. As for II but using Brown Ribbon pectin. The pH after autoclaving was consistently 6·2, probably as a result of demethylation.

The media were poured 3–4 mm thick in Petri dishes which were then dried at 45° and spot inoculated in triplicate with not more than 4 cultures per plate, and incubated at 27°. After 3 days one plate was flooded with

5N hydrochloric acid, the second with 10% (w/v) calcium chloride and the third with 5% (w/v) lead acetate.

IV. PROTOPECTINASE (PP): Lack of knowledge about protopectin is well reflected in the crude methods used for estimating protopectinase activity. Harter and Weimer (1921a and b, 1923) used raw sweet potato discs 1 mm thick suspended in the enzyme solution, and the activity of the solution was taken as the inverse of the time required for the tissue to lose coherence. Coherence was assumed to be lost when the discs offered no perceptible resistance to a pulling stress. Davison and Willaman (1927) standardised the pulling stress by clamping a 10g weight to potato slices 0·5 mm thick immersed in the solution. Ceponis and Friedman (1959) observed the time required for a blunt needle (diameter 0·032 in., with a total weight of 17g) to penetrate lettuce head tissue 400μ thick in an enzyme solution.

Preliminary tests with *E. carotovora* and *X. campestris* showed that there was considerably less PP activity when these cultures were grown and tested in liquid media than when grown on agar media and tested under aerobic conditions. The following method was therefore used: Fresh potato tissue slices 15 mm × 4 mm × 0·5 mm were cut aseptically by microtome. The potato variety "Golden Wonder" was preferred since it gave firm white slices which did not darken rapidly. By pouring 20 ml of 1·5% water agar into sterile Petri dishes (internal diam. 88 mm) a layer 3 mm thick was obtained. Two parallel trenches 11 mm wide were cut in the agar, 28 mm apart. Slices were then laid on the agar 4 mm apart and after approximately 1 min were slid outwards over the trench until they extended unsupported for 7 mm. Thus there were 16 slices in each plate and inoculations were made across each cantilevered slice at the point where it left the support of the agar (see Fig. 1). Loss of strength which causes the unsupported end of the slice to drop to the glass can be an early indication of protopectinase activity but it may be due to loss of cell turgor. Slices were examined at 15 min intervals and the time at which a dropped slice broke easily when tested with a flexible sterile wire was recorded as the maceration time.

Although a preliminary test showed that maceration time decreased with increase in temperature from 17°–37°, it was found most convenient to carry out these tests on the laboratory bench where the temperature was approximately 21°.

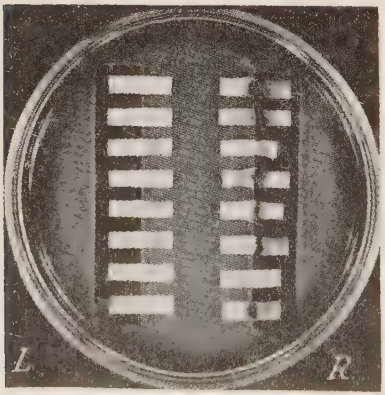

FIG. 1—The cantilever potato slice method for quantitatively testing the protopectinase activity of bacterial cultures. Left: slices uninoculated. Right: inoculated slices in various stages of maceration.

RESULTS

1. LIQUEFACTION: A summary of results is shown in Table 1.

TABLE 1—A Comparison of the Pectolytic Activities of Xanthomonads

Culture (Code Number)	LIQ[n]	PME*	PG**	PP†
G13, G14, i.e., *Erwinia carotovora*	++++	++ 1	abcd	+++
Q1	+++	++ 10	abc	+
Q2, Q19	+++	++ 14	abc	++
Q5, Q9, Q11, Q15, Q16	+++	+ 4	abc	+
Q6, ZG4, ZM1, ZT1	+++	++ 3	abc	++
Q7, Q12–Q14, Q17, Q18	+++	− 24	abc	+
Q10, R1–R5, R9	++	− 24	abc	+
R6, R8, X7, X8, YF1, YF2, ZC1, ZC3, ZJ1, ZJ2, ZR2, ZR3	+	− 24	abc	+
X1–X5	+	+ 4	abc	+
Y2, Y5, Y6, ZF1	−	− 24	a	+
YB1, ZI1–ZI3	+	− 24	abc	++
YB2	+++	− 24	abc	++
YE1, ZR1	−	− 24	abc	−
YG1, YG2	++	− 24	a	+
YH1, ZA3, ZA5	−	− 24	a	−
YI1	+	+++ 3	abc	+
ZA1	−	++ 3	a	−
ZC2	+++	++ 10	abcd	+
ZC4	+++	− 24	abcd	++
ZD1	−	++++ 2	abc	+
ZE1, ZK4	−	−24	abc	+
ZH1	+++	−24	abc	−
ZM2, ZM3, ZU1	−	+++ 3	abc	−
ZP1	−	+ 3	−	−
ZS1–ZS3, ZS5	−	++ 7	a	−
ZS4	+++	−24	abc	++
ZS6	−	++ 14	ab	−
ZX1	++	− 24	abcd	+
All remaining cultures— (i.e. S1–S15, T1–T13, Z1, Z2, YA1, YA2, YC1, YD1, ZE2, ZG1–ZG3, ZG5–ZG7, ZK1–ZK3, ZK5, ZN1, ZN2, ZO1–ZO4, ZQ1–ZQ13, ZS7, ZS8, ZV3, ZY1–ZY3)	−	− 24	−	−

* Pectin methylesterase. Figures refer to days required for reaction.
** Pectin polygalacturonase. Summary from low methoxyl pectin (Rotband). Letters a–d refer to stages of hydrolysis described in Table 2 and shown in Figs 2–5.
† Protopectinase determined with cantilever potato slices.

+++ Maceration within 3 hr at 21°.
++ Maceration 3–5 hr.
+ Maceration 5–15 hr.
− No maceration within 24 hr.

2. PECTIN METHYLESTERASE: Table 1 shows the results and the time required by cultures to produce PME. This test was found to be very sensitive to variations in the conditions of culture, but when these were controlled the method was dependable. The pH of the test solution was found to be critical as the action failed to occur if the initial pH was below 7·0. In order to show whether the comparatively slow production of PME by cultures Q1, Q2, Q19, and ZC2 was due to mutation, further tubes of growth medium were inoculated with one drop of the original cultures after they had been

incubating for 14 days (at which stage all were showing PME). It was found that the secondary cultures still required 10–14 days to produce pectin methylesterase. This suggests that no mutation was involved but that the activity was merely slow in appearing.

TABLE 2—Suggested Breakdown of Pectin and Pectate in Agar

Petri Dish	Developed With	Precipitate	Clear Zone
1	Hydrochloric acid (5N)	Pectic acid	Products simpler than pectic acid (a)
2	Calcium chloride (10%)	Intermediate uronides	Lower uronides and/or galacturonic acid (b)
3	Lead acetate (5%)	Simple uronides (c)	Galacturonic acid or simpler products* (d)

*This refers to the inner zone of clearing. A clear zone was also observed surrounding the white zone of simple uronides but the significance of this outer zone was not determined.

3. PECTIN POLYGALACTURONASE: Using information given by Dingle *et al.* (1953) the reactions expected from flooding the pectin agar plates with hydrochloric acid, calcium chloride, or lead acetate would be as shown in Table 2. Figures 2, 3, 4, and 5 show the various reactions that were obtained by using Rotband pectin agar. Table 3 gives the results of the action of the cultures on the three pectin agar media. A part of this table is included in Table 1 for comparison with other pectolytic activities.

4. PROTOPECTINASE: The results are shown in Table 1.

TABLE 3—Polygalacturonase Activity of Cultures on Three Pectin Agar Media

Culture	Brand of Pectin* A.S.P.	Rotband	Brown Ribbon
G 13, G14, ZC2, ZC4	abcd	abcd	—
Q1, Q2, Q5, Q6, Q9–Q11, Q18, Q19, R1–R9, X1–X8, YB1, YB2, YE1, YF1, YF2, YG2, YI1, ZE1, ZG4, ZH1, ZI1, ZJ1, ZJ2, ZK4, ZM1, ZR2, ZR3, ZS4	abc	abc	—
Q7, Q12–Q17, ZI2, ZI3, ZT1	abc	abc	ab
Y2, Y5, Y6, YH1, ZC3, ZF1, ZS1–ZS3, ZS5	a	a	—
YG1	abc	a	—
ZA1, ZA3	—	a	abc
ZA5	—	—	●
ZC1	abc	abc	ac
ZD1, ZU1	abc	ab	a**
ZM2, ZM3	a	abc	a**
ZR1	ab	abc	—
ZS6	a	abc	—
ZX1	abc	abcd	—
All remaining cultures (i.e., S1–S15, T1–T13, Z1, Z2, YA1, YA2, YC1, YD1, ZE2, ZG1–ZG3, ZG3–ZG7, ZK1–ZK3, ZK5, ZN1, ZN2, ZO1–ZO4, ZP1, ZQ1–ZQ13, ZS7, ZS8, ZY1–ZY3	—	—	—

* = letters refer to stages of hydrolysis shown in Table 2
** = reaction preceded by precipitation of pectic acid as 5–10 mm wide band
— = no action
● = not done

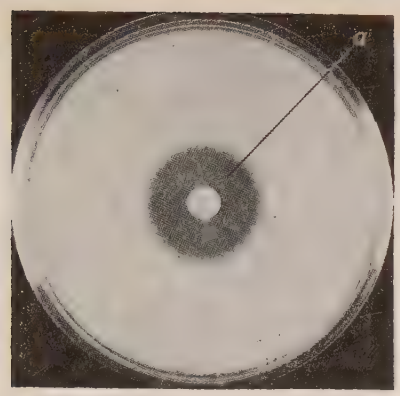

FIG. 2—Hydrolysis of pectin: Rotband pectin agar developed with hydrochloric acid; cf. (a) in Table 2.

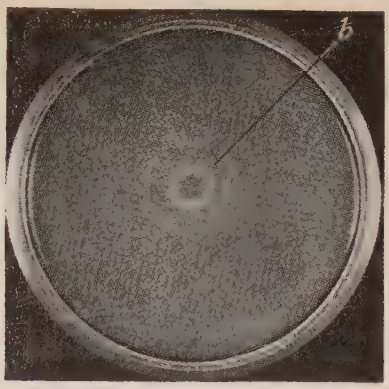

FIG. 3—Hydrolysis of pectin: Rotband pectin agar developed with calcium chloride; cf. (b) in Table 2.

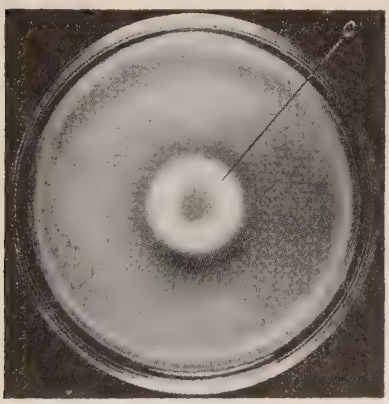

FIG. 4—Hydrolysis of pectin: Rotband pectin agar developed with lead acetate; cf. (c) in Table 2.

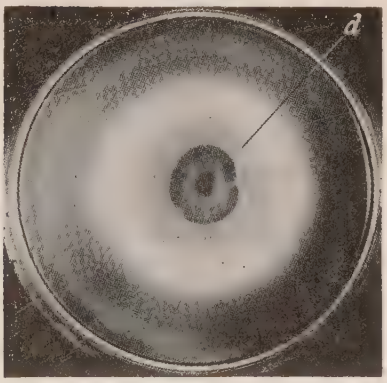

FIG. 5—Hydrolysis of pectin: later stage of Fig 4 reaction; cf. (d) in Table 2.

DISCUSSION

1. LIQUEFACTION: Those cultures causing moderate to strong liquefaction could probably be regarded as having pectin liquefying enzymes, but weak liquefaction could well have been caused by other factors. According to Kertesz (1951) a non-enzymic gel softening or liquefaction can be caused by a degradation of pectic acid at high pH or by peroxide plus ascorbic acid. The results show that there was considerable variation in the amount of liquefaction caused by different cultures of some species. The fact that *X. begoniae* did not liquefy the gel (cf. Sabet and Dowson, 1951) is considered of no significance in view of the variation occurring in other species. Due to this variation it is considered that this test is of little value for the differentiation of xanthomonads.

2. PECTIN METHYLESTERASE: Many cultures which were active gel liquefiers showed no PME reaction, and the reverse was also true. *X cassava* (ZD1) was notable in this latter respect. Smith (1958a) stated that *X. campestris* produces PME, and this was found to be true for 9 cultures. However, 7 further cultures of this species failed to show PME activity in 24 days. From a number of tests made, including pathogenicity tests, there is no reason to believe that these latter cultures are not *X. campestris*. Other species showed similar variation. Therefore it seems either that the conditions of culture were unsuitable for the production of PME, or that the occurrence of this enzyme is variable. Whatever the reason for the variation, the detection of PME, at least by the method used in this work, is of no value for differentiating xanthomonads. The results suggest that PME production is not closely associated with pectin gel liquefaction, pectin polygalacturonase or protopectinase.

3. PECTIN POLYGALACTURONASE: The results show that a number of xanthomonads possess enzymes of the polygalacturonase type. However, if pectin polygalacturonase is deemed to be present only if the hydrolysis proceeds to monogalacturonic acid, whereas a less complete hydrolysis of low methoxyl pectin is due to pectin depolymerase, then it would appear that both enzymes were demonstrated among the organisms. Further work with these cultures and more information about PG and DP is required before the results can be fully interpreted. Polygalacturonase activity may be of interest in relation to pathogenicity but due to the variation observed within the species and between media, the property appears to be of little value for the identification of xanthomonads.

4. PROTOPECTINASE: In view of the results obtained it seems possible that enzymes able to cause the disintegration of tissue may have a more important role in infective processes by xanthomonads than has previously been recognised. *X. campestris*, which produces a vascular browning and leaf spot necrosis of crucifers, is often associated with a soft-rot commonly attributed to *Erwinia carotovora*, e.g. cultures Q2 and Q6 could well cause a soft-rot in the field similar to that which they induce in tissue slices. Similarly, the soft wet rot that frequently occurs in hyacinth yellows may not be due to a secondary infection by *E. carotovora* but to *X. hyacinthi* (see cultures Y2, Y5, Y6) alone. However, the ability of an organism to secrete protopectinase in pure culture may be quite unrelated to its ability to invade and parasitise plant tissues. In certain cases the organism may not grow well in the living plant, or if it does so, nutrient conditions may not be favourable for enzyme secretion. Also, any enzymes which are secreted may be absorbed or inactivated by the plant tissue or tissue sap (Wood, 1955). Possibly such explanations provide the reason why most *Xanthomonas* cause only local lesions in plants, regardless of whether they are able or not to secrete protopectinase.

Table 1 shows that no direct relationship was demonstrable in these experiments between PP activity and PME, PG or liquefaction of a pectate gel. It also shows that due to strain variation, little use can be made of the presence or absence of protopectinase for the identification of species cf. cultures of *X. malvacearum* (ZG1–ZG7), *X ricinicola* (ZM1–ZM3), *X. uppalii* (ZR1–ZR3), and *X. vasculorum* (ZS1–ZS8).

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A SIMPLE CIRCULATING PUMP FOR LIQUIDS

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A simple, economic circulating pump, which was designed to stir a thermostatically controlled water bath and circulate the liquid to external apparatus, is described. This type of pump does not seem to be well known and only one description of a similar pump could be found in the literature (Cunliff, 1923).

One design, capable of circulating liquid at a constant head of pressure from a thermostatically controlled bath is shown in Fig. 1 (A). This pump uses a well known type of centrifugal stirrer consisting of a bulb A constructed from glass tubing, pierced with three or four holes (1 to 2 mm in diameter) and spun at high speed in the liquid. The shaft of this stirrer is neatly fited into a rubber bung B and this seal is, of course, lubricated by the liquid in the bath. For some work this bung could be of teflon which is not affected by many liquids and will stand elevated temperatures. A stirring propeller S may be fitted to this shaft if desired. A small stirring motor is connected to the glass shaft by a rubber coupling C. A rubber bung D may be needed to prevent air from being sucked into the pump. With a bulb 2 cm in diameter spun at 1,000 revs/min by a 1/60 H.P. motor, over 1½ litres of water per minute could be circulated to external apparatus. One stirrer in this laboratory has given several months of continuous, trouble-free operation.

Another design (Fig. 1 (B)) which circulates liquid at a higher pressure was also found to work satisfactorily.

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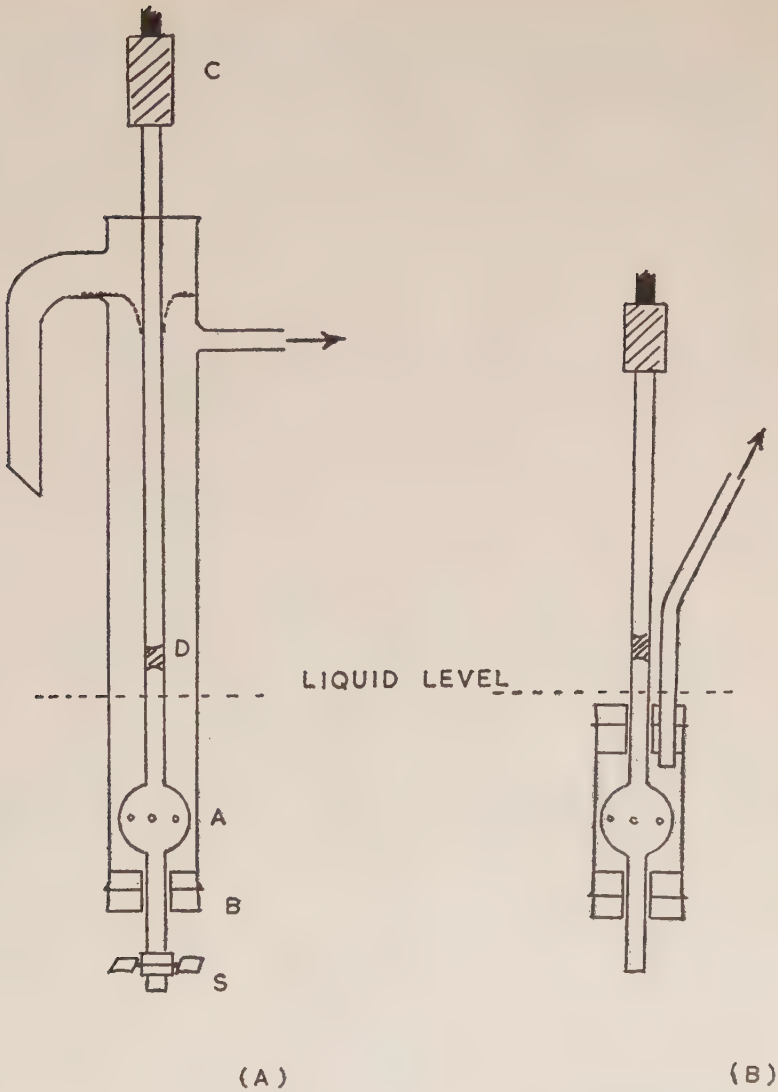

FIG. 1—Diagram of circulating pump

A NOTE ON THE BIOLOGICAL ACTIVITY OF THE ISOMERS OF BIOTIN HOMOLOGUES AND THEIR SULPHONES

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Stokes and Gunness (1945) have reported that *l*-biotin cannot replace *d*-biotin for the nutrition of *Lactobacillus casei* or *Lactobacillus arabinosus* in pure culture. This observation was confirmed by the finding that *dl*-biotin has only 50% of the activity of *d*-biotin for the above lactobacilli and yeast (Baker *et al.*, 1947, Stokes and Gunness, 1945). Rubin *et al.* (1945), and Wood and du Vigneaud (1945) have prepared various dethiobiotins and report that for the growth of yeast *dl*-dethiobiotin and *d*-dethiobiotin have equal activity with *dl*-biotin and *d*-biotin respectively. These results demonstrate that the *l*-isomers of biotin and dethiobiotin are biologically inert.

The two homologues of biotin, homobiotin (caproic acid side-chain) and norbiotin (*n*-butyric acid side-chain), and the corresponding sulphones have been shown by Goldberg *et al.* (1947), and Rubin and Scheiner (1949), to be biotin antagonists for the growth of a number of micro-organisms. These investigations, however, were carried out with the racemic compounds and it was unclear whether the anti-biotin properties were confined to one isomer. During studies by the author and H. H. Williams of the effects of biotin and some of its analogues on yeast glycolysis, pure samples of the *d*-isomers of homobiotin, norbiotin and the two sulphones were prepared, and it was decided to determine the molar inhibition ratios (MIR) of these compounds for a number of micro-organisms. The MIR is defined as the moles of analogue that will inhibit the effects of one mole of *d*-biotin, and is determined by measuring the amount of analogue required to reduce the growth of a micro-organism on a synthetic medium containing 0·2 mγ of *d*-biotin per 10 ml to a growth equivalent to 0·1 mγ per 10 ml.

The organisms used in the study were *L. arabinosus* 17–5, *L. casei* epsilon, and *Saccharomyces cerevisiae* 139, all of which were obtained from the American Type Culture Collection in Washington, D.C. Growth of the lactobacilli was determined by acid production after incubation for 72 hr at 30°C, and of the yeast by measurements of optical density in matched spectrophotometer tubes after incubation for 20 to 22 hr at 37°C.

The MIR of the four analogues for each micro-organism is given in the Table. A comparison of these figures with the MIR for the racemic compounds (Goldberg *et al.* 1947, and Rubin and Scheiner, 1949) clearly demonstrates that the *d*-isomers of these analogues are approximately twice as effective antibiotins as the *dl*-isomers. It is therefore concluded that the *l*-isomers of homobiotin, norbiotin, homobiotin sulphone, and norbiotin sulphone are biologically inert. Combined with the previous studies of the activities of biotin and dethiobiotin, this conclusion illustrates the absolute stereospecificity of the biotin molecule in biological systems.

TABLE 1—Molar Inhibition Ratios

Analogue	*L. arabinosus**	*L. casei*†	*S. cerevisiae*‡
d-homobiotin	2000	60	260
d-norbiotin	100000	11000	470
d-homobiotin sulphone	600	210	20000
d-norbiotin sulphone	100000	30000	100000

*Medium of Wright and Skeggs, 1944.
†Medium of Shull, *et al.*, 1942.
‡Medium of Hertz, 1943.

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EXPERIMENTAL FLUIDIZED-BED REDUCTION OF NEW ZEALAND IRONSANDS

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(Received for publication, 18 December 1959)

Summary

Experiments with a small spouted-bed reactor have shown that fluidized-bed reduction of New Zealand titaniferous ironsands with hydrogen, and with carbon monoxide containing 30% hydrogen, can be conducted without sticking, in the temperature ranges 700°–900°C. At 900°C reduction proceeds rapidly to a high degree of metallisation.

Fluidized-bed reduction with carbon monoxide is possible, without sticking, in the temperature ranges 600°–750°C, but reduction rates are relatively low.

Preoxidation of the ironsands increases the rate of subsequent reduction with carbon monoxide and/or hydrogen, the effect of preoxidation being particularly marked at the lower reduction temperatures.

Rates of metallisation and gas utilisations in the reduction of titaniferous ironsands have been determined and compared with similar data for a purer grade of magnetite. These comparisons indicate that the impurities present retard the reduction of the titaniferous ironsands but extend the temperature ranges at which reduction can be effected without sticking.

INTRODUCTION

In recent years considerable attention has been devoted to the direct reduction of iron ores (Barrett, 1954; Cavanagh, 1958; Wright, 1957; Communauté Européenne Du Charbon et De L'acier Haute Autorité, 1958) including fluidized-bed methods of accomplishing reduction with carbon monoxide and/or hydrogen (Stelling, 1958; Kuzmick and Bruland, 1958; McQuaid, 1958; Squires and Johnson, 1957). Factors controlling the technical efficiency of such processes include reactivity of the ore with carbon monoxide and/or hydrogen, operating temperature, and in the case of fluidized-bed reduction, the sticking properties of the ore in fluid beds. Published data (Stelling, 1958; McQuaid, 1958) suggest that the reactivity and sticking properties may be characteristic of both type and source of ore, being governed by the physical characteristics of the ore and by the mineralogical constitution of the ore minerals and gangue constituents.

The gaseous reduction or direct reduction processes are of considerable interest to the utilisation of New Zealand titaniferous ironsands for steel production, using non-coking coal and electric power (Marshall, 1959). However, no published data are available on the reactivity and sticking propensities of these ores. The present investigations were therefore undertaken to obtain information on these properties of New Zealand ironsands, so that some assessment could be made of their suitability for treatment by the modern direct reduction processes.

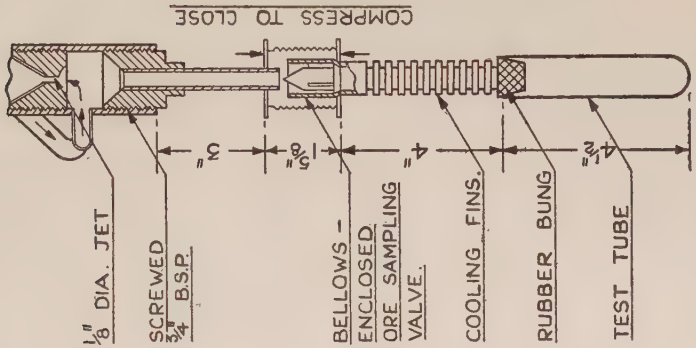

FIG. 1 (b). Details of ore sampler.

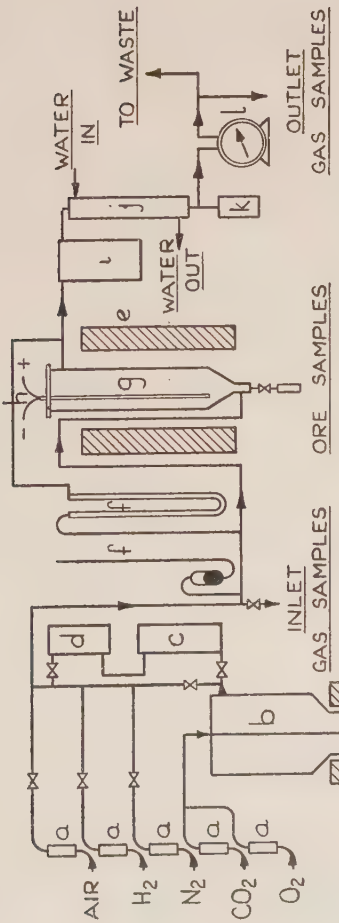

FIG. 1 (a) Equipment layout.

EXPERIMENTAL

Equipment

Figure 1 (a) shows the layout of the apparatus used, which consisted essentially of a spouted-bed reactor (Mathur and Gishler, 1955) with the usual auxiliary equipment for measuring bed temperatures, pressures and gas flowrates, together with a generator for producing carbon monoxide.

The only non-standard items were the carbon monoxide generator and the spouted-bed reactor. The carbon monoxide generator consisted essentially of a hopper which supplied a continuous feed of charcoal to a furnace held at 1,100°C. A mixture of carbon dioxide and oxygen was passed through the hot charcoal bed to generate carbon monoxide by the reactions $CO_2 + C = 2CO$, $2C + O_2 = 2CO$. The resultant gas, carbon monoxide containing 6–12% of carbon dioxide, with an average content in each run of 10% carbon dioxide, was fed continuously to the reactor. For comparison purposes a few runs were conducted with purer carbon monoxide, obtained by scrubbing the 10% carbon dioxide gas with caustic soda solution and drying it with calcium chloride. This gas contained 1·4 to 3% of carbon dioxide, the average content being 2%.

The decision to use a spouted bed reactor was made after preliminary empirical tests had shown that, in a small reactor, this type of bed could be easily maintained in a fluidized state over the pressure and wide temperature ranges desired, and that a simple reactor of this type was very robust and easy to construct.

Inconel sheet was used to fabricate the final reactor which was $3\frac{1}{2}$ inches in diameter, 19 inches long, and had a 60° bottom cone with a $\frac{1}{8}$-inch diameter jet. Under this jet a valve and sample tube were mounted (Fig. 1 (b)) to enable samples of the ironsand to be taken during a run, simply by checking the gas flow for a short time to allow bed material to flow out through the spouting jet.

Materials Used

GASES

Air used in oxidation runs was supplied by a small compressor; all other gases, including the carbon dioxide and oxygen used in forming carbon monoxide, were drawn from commercial high-pressure cylinders.

SOLID MATERIALS

Coconut charcoal was employed as the source of carbon for the carbon monoxide generator. The ironsands used were concentrates from Patea, Taharoa, and Gillespie's Beach. Patea ironsand was used for most of the tests since it was the most readily available. Taharoa and Gillespie's Beach ironsands were used for comparisons with the Patea material. Table 1 gives typical chemical and size analyses for these ores. When oxidised ironsands were required, oxidation with air was conducted in the spouted-bed reactor at 1,000°C for $1\frac{1}{2}$ hr, the product being passed through a magnetic separator to remove any incompletely oxidised particles. This treatment gave oxidised ironsands containing less than 1% ferrous iron.

TABLE 1—Chemical and Sieve Analyses of Ironsand Concentrates

	Patea Ironsand Concentrates		Taharoa Ironsand Concentrates		Gillespie's Beach Ironsand Concentrates	
	Unoxidised	Oxidised	Unoxidised	Oxidised	Unoxidised	Oxidised
CHEMICAL ANALYSES						
Total Fe%	57·5	56·8	53·2	49·1	69·8	66·8
FeO%	28·4	< 0·1	26·1	< 0·1	30·0	< 0·1
Fe_2O_3%	50·6	81·2	47·1	70·2	66·5	95·5
TiO_2%	8·2	8·0	7·4	6·7	1·6	1·6
Al_2O_3%	4·6	4·0	5·0	4·6	1·1	1·0
MgO%	3·0	3·2	4·0	4·2	< 0·1	< 0·1
MnO%	0·67	0·67	0·64	0·61	0·06	0·06
CaO%	1·2	1·0	2·6	3·4	0·5	0·4
P_2O_5%	0·43	0·34	0·44	0·42	0·11	0·07
V_2O_3%	0·37	0·41	0·32	0·32	0·17	0·21
SiO_2%	2·3	0·5	6·8	8·7	0·4	0·3
Total	99·8	99.3	100·4	99·2	100·4	99·1
SIEVE ANALYSES*						
+ 25	0·0%		0·0%		0·0%	
— 25 + 36	0·2%		0·2%		0·2%	
— 36 + 52	0·2%		1·2%		4·5%	
— 52 + 72	0·5%		8·3%		35·8%	
— 72 + 100	6·3%		35·0%		53·5%	
— 100 + 150	73·5%		46·5%		6·0%	
— 150 + 200	11·3%		5·5%		.....	
— 200	8·0%		3·3%		.....	

*British Standard Specification Sieves.

Operating Methods

OXIDATION TESTS

4·5 lb of sand were loaded into the reactor, fluidized with nitrogen, and heated until the desired temperature was attained. Air at 0·25 cu. ft/min was then substituted for the nitrogen. Samples were drawn at 10, 20, 40, 80, 120 minutes from the time of switching to air. The samples were analysed chemically for ferrous iron, and the degree of oxidation calculated using the formula:

$$\text{Degree of oxidation} = \frac{\%\ \text{FeO in ore} - \%\ \text{FeO in product}}{\%\ \text{FeO in ore}} \times 100$$

Air oxidation tests were carried out at temperatures of 700°, 850°, and 1,050°C on Patea ironsand; further runs were conducted on the same ironsand using oxygen-nitrogen mixtures to determine the effect of varying the partial pressure of oxygen.

CARBON MONOXIDE REDUCTIONS

The same initial procedure as that used for the oxidation runs was employed, except that carbon monoxide containing 10% of carbon dioxide was substituted for the nitrogen when the desired temperature was reached. A

charge of 4·5 lb of ironsand and a gas flowrate kept as close as possible to 0·25 cu. ft/min were used for all of these runs. Gas samples from the inlet and outlet gas streams of the reactor were taken at intervals throughout the run for analysis. By plotting the difference between the percentages of carbon dioxide in inlet and outlet gases against time, curves indicating the degree of gas utilisation were drawn. Since the gas flowrate was known, the progress of reduction could be followed during a run by measuring the area under this utilisation curve, provided no carbon deposition had occurred by the reaction $2CO = CO_2 + C$ (Stelling, 1958). For most of these experiments an arbitrary maximum time of 8 hours was used since it was considered that any longer period would not have much practical significance.

Ironsand samples were withdrawn from the reactor at intervals throughout each run, and were allowed to cool under carbon monoxide in the sampling tube before analysis. These samples were examined metallographically and analysed chemically for total iron, metallic iron, and in some cases for carbon content. Some X-ray diffraction analyses were also made to verify the presence of iron carbide. Since the determination of ferrous and ferric oxides in the presence of metallic iron is difficult and of questionable accuracy, and since total oxygen determinations are not sufficiently rapid for routine analyses, no attempt has been made to plot the percentage removal of oxygen from the ironsands. Instead, the formation of metallic iron has been plotted, the degree of reduction finally attained being shown at the end of each curve. The following method was used to calculate the degree of reduction:

$$\% \text{ reduction} = \frac{100}{x}\left[\frac{100\,(x - y)}{100 - y}\right]$$

where $x = \%$ oxygen combined with iron in the ironsand at the start of the run, calculated from the ferrous and ferric iron analyses.

$y = \%$ oxygen combined with iron in the reduced sample, calculated from the total iron and metallic iron contents, assuming that iron is present as only metallic iron and ferrous oxide.

Since chemical analysis for iron carbide in the presence of free carbon and iron oxide is also difficult and of questionable accuracy, products containing iron carbide (from carbon monoxide reductions at 600°C) were heated in flowing oxygen-free argon for 20 minutes at 900°C prior to analysis. This treatment was employed to decompose part or all of the iron carbide according to the reaction FeO (residual) + $Fe_3C = 4Fe + CO$, which proceeds rapidly at temperatures above 700°C (Stelling, 1958); the argon flow was used to prevent reoxidation of the metallic iron, and the limited heating time to minimise the effects of possible side reactions with carbon monoxide and with free carbon in the products, according to the equations, $CO + FeO = Fe + CO_2$ and $C + FeO = Fe + CO$.

After heating in this manner the products were analysed for metallic iron and total carbon, the results being reported as "equivalent metallic iron" and "residual carbon" respectively. The progress of carbon monoxide reductions at 600°C have been followed by plotting "equivalent metallic iron" and "residual carbon" contents against reduction time, as in Fig. 2. Reduction

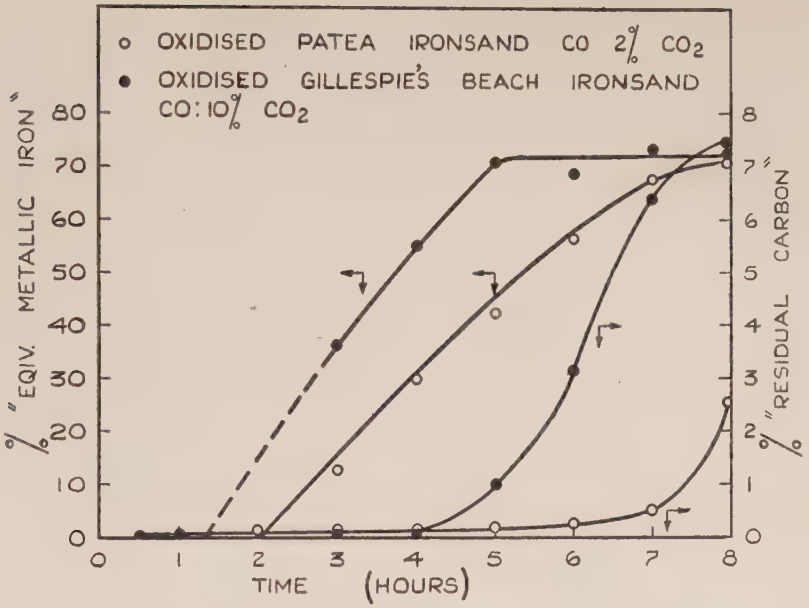

FIG. 2—Rate of reduction; carbon monoxide at 600°C.

under these conditions is considered to be complete when sufficient iron carbide is present to react stoichiometrically with the residual iron oxide, leaving no excess of iron oxide and no excess of carbon, i.e. when the "equivalent metallic iron" content reaches the theoretical maximum value.

This empirical method of following the progress of carbon monoxide reduction at 600°C is considered valid since, in any commercial utilisation, products containing iron carbide would be treated in a similar manner by heating to temperatures above 700°C (Stelling, 1958).

HYDROGEN REDUCTIONS

Again the same preliminary procedure was adopted, using the same weight of ironsand, 4·5 lb. Hydrogen at 0·44 cu. ft./min. replaced the nitrogen when the required temperature was attained. Ironsand samples were taken from the bed at intervals during each run and left to cool under hydrogen before being removed. These samples were examined metallographically and analysed for total and metallic iron.

The progress of these runs was followed by measuring the rate of water production and the gas flow simultaneously at regular intervals. The cumulative total water formation indicated the amount of reduction achieved. From these data the partial pressure of water vapour in the gas from the reactor was calculated, to enable gas utilisation curves to be plotted.

MIXED CARBON MONOXIDE AND HYDROGEN REDUCTION

The same preliminary procedure was again adopted, the 4·5 lb charge of ironsand being brought up to temperature while fluidized with nitrogen. The nitrogen was then replaced by a gas mixture containing 29·5% hydrogen, 70% carbon monoxide, and 0·5% carbon dioxide at a total flowrate of 0·26 cu. ft./min.

Ironsand samples were withdrawn periodically and cooled under the reducing gas before being removed for analysis.

ANALYTICAL METHODS

Total iron determinations were carried out by the standard method (Lundell, Hoffman, and Bright, 1931) of dissolving the sample in hydrochloric acid followed by reduction with stannous chloride solution and titration with potassium dichromate solution. The ferrous iron content was determined by dissolving the sample in hydrochloric acid and titrating with potassium dichromate solution under an atmosphere of carbon dioxide, to prevent oxidation (Lundell, Hoffman, and Bright, 1931). For determinations of metallic iron the mercuric chloride method (Barrett, 1954) was used. Total carbon determinations were conducted by the standard combustion method (Pigott, 1953) with volumetric measurement of carbon dioxide in a Strohlein-type apparatus.

Gas samples were analysed by the Orsat and Bone Newitt methods (Vogel, 1941). X-ray identification of products in the powdered, reduced samples was carried out in a Norelco Counter Diffractometer, with chromium $K\alpha$ radiation.

For metallographic examinations ironsand samples were mounted in methyl-methacrylate moulds by the normal metallographic method. Sections were prepared by wet grinding with 600 mesh abrasive paper, followed by polishing with 4—8μ and 0—1μ diamond powders. Metallic iron or iron carbide could be easily differentiated from magnetite or wüstite, and from hematite by visual examination of the unetched specimens on a metallographic microscope. Wustite and magnetite were differentiated by etching with 1% hydrochloric acid in alcohol. Metallic iron and iron carbide were differentiated by means of the usual carbide metallographic etchants (Lyman, 1948).

RESULTS

Oxidation

As shown in Fig. 3 the Patea ironsand can be oxidised rapidly and completely in air at 1,050°C. At 850°C the oxidation rate was low, and only 60% oxidation was attained in 2 hours; At 700°C 24 hours was required to achieve 60% oxidation.

Sticking, sintering, and the formation of small agglomerates were encountered at 850° and 1,050°C, being more severe at the higher temperature. Further oxidation runs showed that, at 1,000°C, a mixed gas, containing 50% nitrogen and 50% oxygen, caused more rapid sintering, while 94% nitrogen

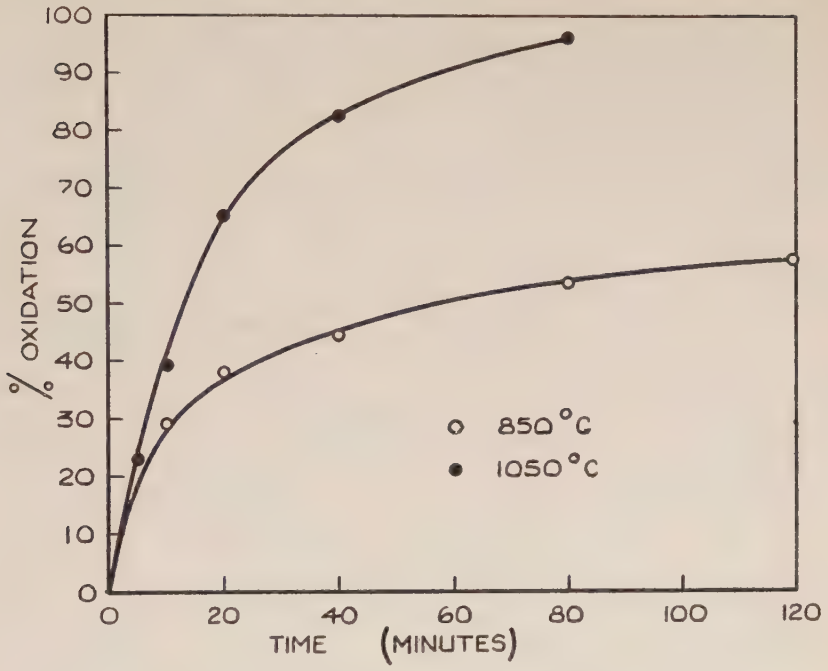

FIG. 3—Rate of oxidation of Patea ironsand with air.

and 6% oxygen reduced, but did not eliminate, the sintering. The sintering troubles were finally overcome by simulating continuous operation of the reactor, small batches of raw sand being fed intermittently into a hot, fluidized bed of preoxidised material, with intermittent removal of the oxidised product to maintain constant bed level.

Carbon Monoxide Reduction

Reduction runs on Patea ironsand (unoxidised) at 600°, 700°, 750°, 800°, and 900°C, using carbon monoxide containing 10% of carbon dioxide, were all characterised by very slow reduction rates. At 600°C no iron carbide or metallic iron was produced in 4 hours. Severe carbon deposition occurred by the reaction $2CO = C + CO_2$, and caused excessive carryover (> 2 oz) of fine carbon in the exit gases so that the run had to be terminated. At 700°C only 2% metallic iron was produced in 8 hours, and again excessive carbon deposition ($> 2{\cdot}5$ oz) occurred. At 750°, 800°, and 900°C, practically no carbon deposition and no sticking of the beds occurred, but reaction rates were very low, with less than 15% metallic iron being formed in 8 hours. The results of the 900°C run are shown in Fig. 4.

To investigate the effect of preoxidation, reduction runs were next conducted on oxidised Patea ironsands at 600°, 700°, 750°, 800°, and 900°C, using the same reducing gas, carbon monoxide containing 10% of carbon dioxide.

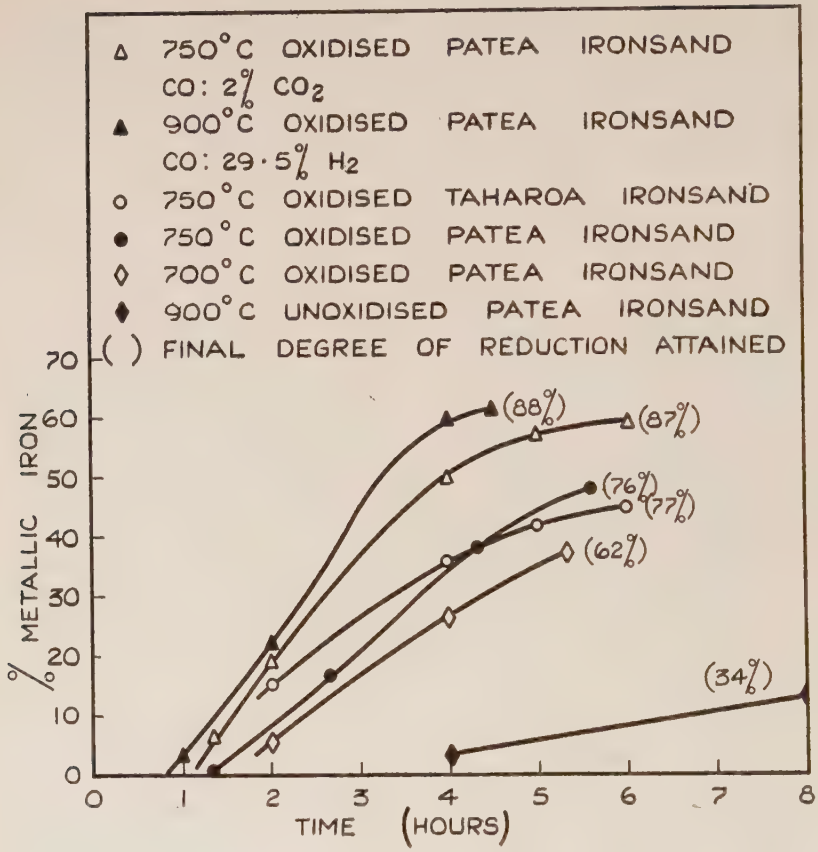

FIG. 4—Rate of formation of metallic iron, carbon monoxide reduction. Input gas composition CO:10% CO_2 except where shown.

At 600°C only small amounts of iron carbide, as indicated by X-ray diffraction and metallographic examination, were formed in 8 hours. Excessive carbon deposition occurred, producing 7·5% total carbon in the reduced material. At 700° and 750°C, reduction to metallic iron occurred with practically no carbon deposition, and no sticking of the bed. The rate of formation of metallic iron at these temperatures is shown in Fig. 4. At 800° and 900°C, sticking of the beds occurred at the time when metallic iron was starting to form from wüstite, and forced the termination of the runs.

To check the effect of carbon dioxide concentration in the reducing gas, two further runs were conducted on oxidised Patea ironsands at 600° and 750°C, using scrubbed and dried generator gas, i.e. carbon monoxide con-

taining an average of 2% carbon dioxide. The 600°C run with this gas produced iron carbide. No sticking of the bed was experienced, and only a moderate amount of carbon deposition occurred. After 8 hours reduction the product contained 60% total iron and 9·5% total carbon. After heating at 900°C in argon this product contained 71% "equivalent metallic iron", 72·3% total iron, and 2·6% "residual carbon", equivalent to approximately 98% reduction. The progress of reduction is indicated in Fig. 2. Results of reduction at 750°C of Patea ironsand with carbon monoxide containing 2% carbon dioxide are included in Fig. 4.

By comparison with previous runs using carbon monoxide containing 10% carbon dioxide, the use of purer carbon monoxide (2% CO_2) significantly increased the rates of reduction at 600° and 750°C.

Further comparison runs were carried out on oxidised Taharoa ironsand using carbon monoxide containing 10% carbon dioxide, to indicate whether the source of the material had any major effect on the carbon monoxide reduction of titaniferous ironsand. Reduction at 600°C gave similar results to those obtained with oxidised Patea ironsand, the material being reduced essentially to wustite in 6 hours, with only small amounts of iron carbide as indicated by X–ray diffraction and metallographic examination. Excessive carbon deposition and carryover forced termination of the run.

The results of reduction at 750°C of oxidised Taharoa ironsand are included in Fig. 4, from which it will be noted that the reduction curve shape for oxidised Taharoa ironsand is similar to that of oxidised Patea ironsand at 750°C. With the Taharoa material 77% reduction was attained in 6 hours compared to the 76% reduction for Patea material in 5·3 hours.

To indicate the effects of ore purity on fluidized-bed reduction, runs were conducted at 600° and 750°C, using Gillespie's Beach ironsand, which was the purest magnetite sand available. During reduction of oxidised Gillespie's Beach ironsand at 600°C, with carbon monoxide containing 10% carbon dioxide, rapid formation of iron carbide occurred in the first 5 hours. The rate of carbide formation then decreased, carbon deposition becoming the predominant reaction, and the ratio of iron carbide to residual iron oxide did not reach the stoichiometric value equivalent to complete reduction. After heating at 900°C in argon, the product from 8 hours' reduction contained 72·3% "equivalent metallic iron", 87·6% total iron, and 7·5% "residual carbon", equivalent to approximately 80% reduction. As shown in Fig. 2, carbide formation equivalent to approximately 80% reduction occurred in the first 5 hours, at which time reduction virtually ceased. X-ray diffraction analyses and metallographic examination confirmed the presence of iron carbide in the reaction products. No sticking occurred in this run, but carbon deposition and carryover in the reaction gases was experienced, becoming severe after 6 hours reduction.

During reduction of oxidised Gillespie's Beach ironsand with carbon monoxide at 750°C, sticking was encountered at the stage when metallic iron started to form from wustite, and forced the termination of the run.

Fig. 5 shows typical gas utilisation curves for carbon monoxide reduction runs.

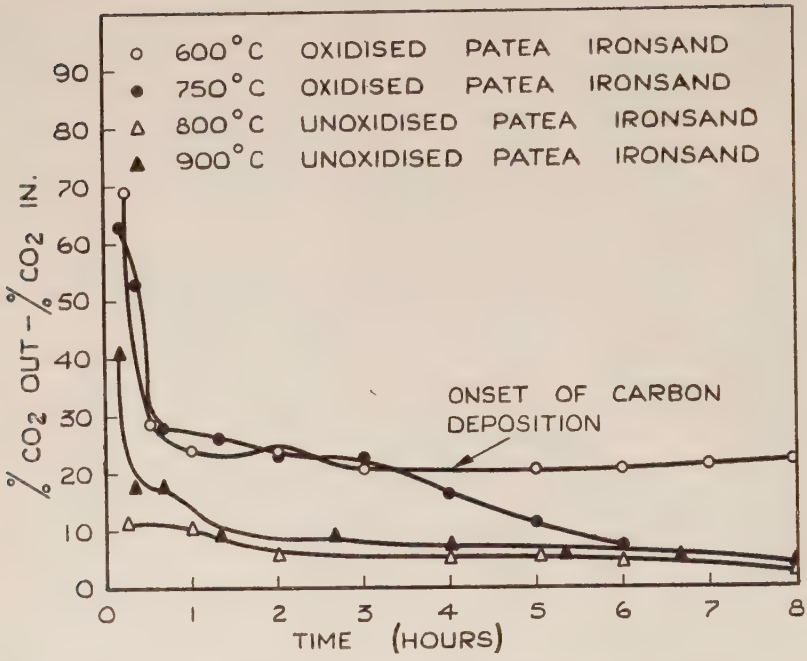

FIG. 5—Gas utilisation, carbon monoxide reduction. Input gas composition for 600°C oxidised Patea ironsand, CO:2% CO_2; for the remainder CO:10% CO_2.

Hydrogen Reductions

As indicated in Fig. 6 reduction of unoxidised Patea ironsand at 750°C with hydrogen was relatively slow, only 36% metallic iron being formed in 5·75 hours. At 900°C reduction proceeded rapidly, 71% metallic iron being formed in 4·5 hours.

To investigate the effect of preoxidation on the reactivity of the ironsand to hydrogen, and to obtain further reaction rate data, hydrogen reduction runs were conducted on oxidised Patea iron sand at 600°, 700°, 750°, 800°, 900° and 1,000°C.

At 600°C reduction proceeded to the stage where metallic iron started to form from wustite, at which point sticking of the bed prevented further fluidization.

The runs with hydrogen at 700°, 750°, 800°, and 900°C gave rapid reduction without sticking of the bed. The rates of formation of metallic iron during these runs are indicated in Figs 6 and 7. Reduction rate increased with operating temperature, the best results being obtained at 900°C when 70% metallic iron was attained in 3·3 hours' reduction. Reduction at 1,000°C proved to be impossible owing to sticking of the bed, which occurred at or near the stage of initial formation of metallic iron.

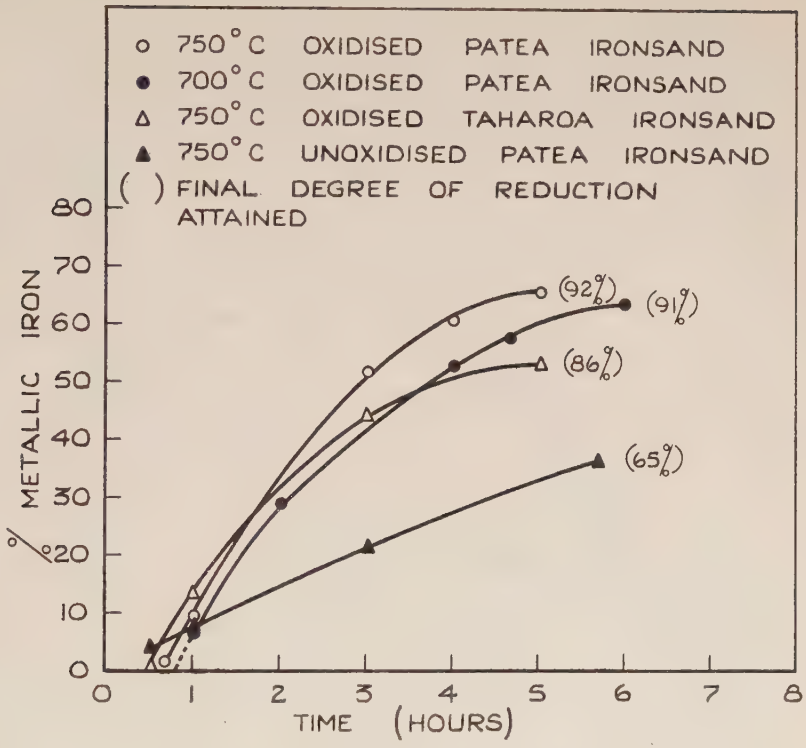

FIG. 6—Rate of formation of metallic iron, hydrogen reduction.

For comparison, oxidised Taharoa ironsand was reduced with hydrogen at 750° and 900°C. The results of these runs, as shown in Figs 6 and 7, indicated that oxidised Taharoa ironsand is slightly less reactive than the oxidised Patea material.

Attempts were made to reduce oxidised Gillespie's Beach ironsand with hydrogen, at 600°, 750°, and 900°C, to investigate the effect of higher ore purity on hydrogen reduction. All of these runs had to be abandoned owing to sticking of the bed which set in at or very near the stage where metallic iron was starting to form from wustite.

Typical gas utilisation curves for hydrogen reduction runs are given in Fig. 8.

Reduction with Mixed Carbon Monoxide and Hydrogen

To determine if the sticking of a fluidized bed with carbon monoxide at 900°C could be eliminated by mixing hydrogen with the carbon monoxide, a further run was conducted using oxidised Patea ironsand fluidized with a gas mixture containing 70% carbon monoxide, 29·5% hydrogen and 0·5%

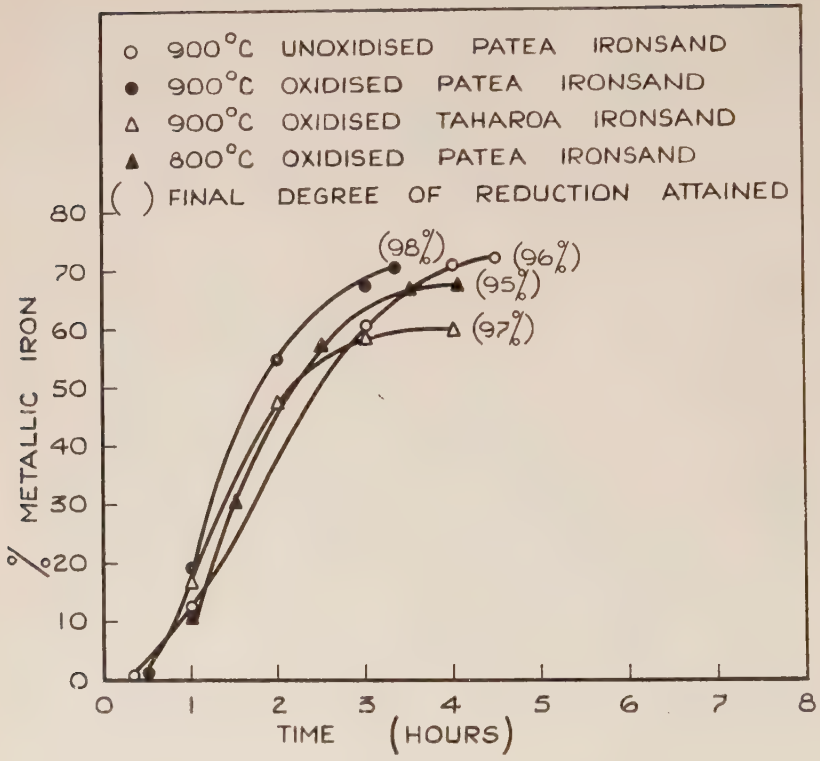

FIG. 7—Rate of formation of metallic iron, hydrogen reduction.

carbon dioxide, at 900°C. No sticking was experienced and only slight carbon deposition occurred. A product containing 60·4% metallic iron and 0·15% carbon was obtained in 4·5 hours. The rate of formation of metallic iron is shown in Fig. 4.

Agglomeration and Sticking

Small agglomerates were noticed in the ironsand when it had been oxidised. They consisted of a few grains of sand clustered together and amounted to 30–45% of the total weight of the ironsand.

During reduction with carbon monoxide at 600°C these clusters were broken down, but at higher temperatures no appreciable alteration in either quantity or size became apparent. With the hydrogen runs, the final product contained about the same proportion of agglomerates, but during the run the proportion rose to a peak at the time when transition from magnetite to wustite was taking place, and then decreased. These clusters were probably formed in the slower moving zones of the bed. As no size increase with

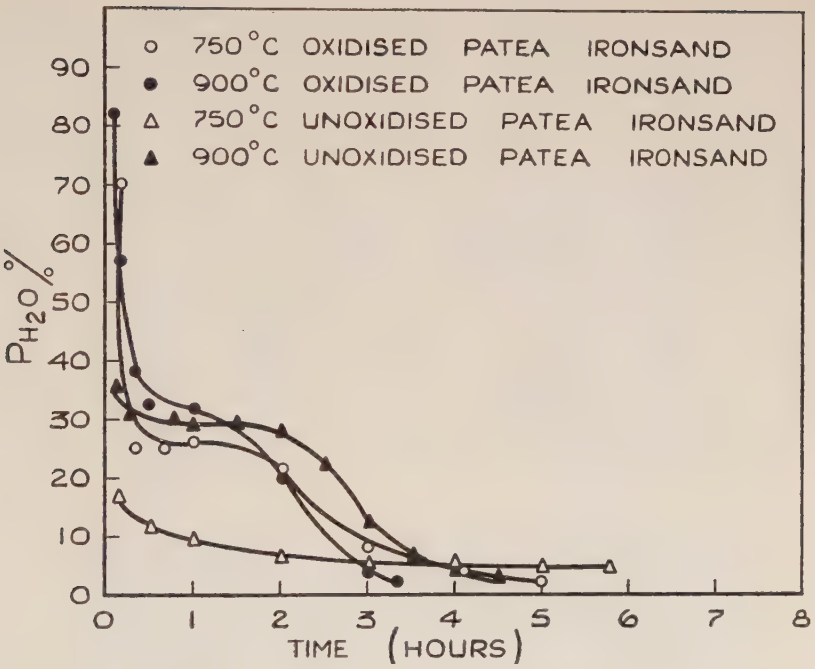

FIG. 8—Gas utilisation, hydrogen reductions.

time was noted, it is probable that the building up of the agglomerates to the size encountered was governed by attrition occurring in the faster-moving material of the spout. Where sticking set in, no prior indication was given by the size and proportion of the agglomerates in samples withdrawn from the bed just prior to sticking.

The sticking action was quite abrupt, highly reproducible, and could not be delayed by simple physical methods such as agitation of the bed, increasing the gas flows, etc. External indications were a drop in bed temperature caused by alteration in heat transmission from the reactor walls, followed by a rapid fall in pressure drop across the reactor, to that of an empty tube. From observations made on an open reactor, a possible sequence of events leading to sticking is as follows. First, at the stage of reduction where conversion to wustite has occurred, and/or metallisation is commencing, the intergranular bonding forces are sufficient to hold the ironsand stationary in the zones close to the walls of the reactor, where at all times the bed movement is slow. This fixed sand then retards the movement of sand adjacent to it so that the stationary zone grows radially inwards until the diameter of the central core is so reduced that the remaining sand is ejected by the gas stream and lies on top of the static bed. The mechanism of the bonding responsible for sticking is not known. Diagrams showing a normal spouted bed and a stuck bed are given in Fig. 9.

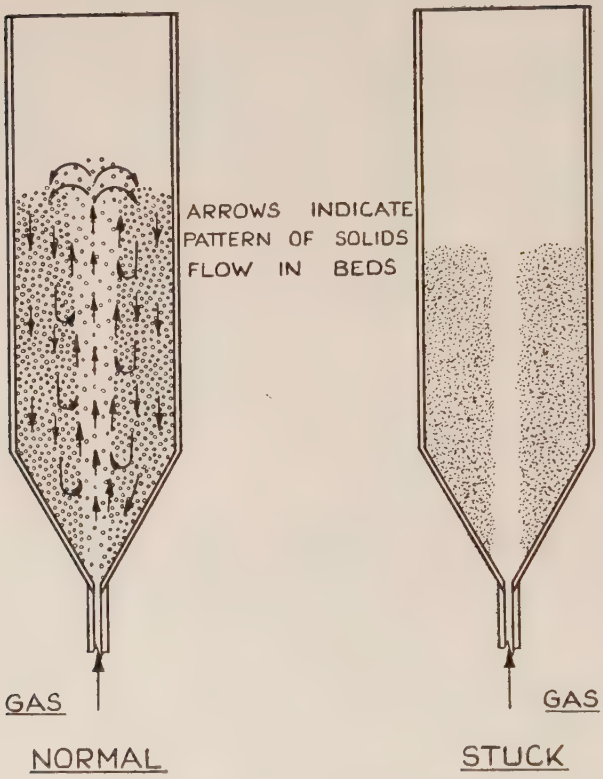

FIG. 9—Schematic diagrams of spouted beds.

A hot, fluidized bed which had become static in this manner behaved like a viscous fluid, and could be stirred with a rod, but when the agitation was stopped the bed immediately became static again, even if the gas flow was increased. When such a bed was cooled to room temperature the ironsand could be rendered free-flowing again simply by disturbing the bed, e.g. by poking it lightly with a rod or by tapping the reactor body; indeed, the bonding forces were so slight that it was difficult to pick up a small lump of the bonded sand without its breaking up. Similar phenomena encountered during the high-temperature chlorination of olivine in a fluid bed have been described by Bengston and Johanson (1959). If a fluid bed which has become static, in the above manner, is not immediately cooled, diffusional bonding of the sand eventually produces a sinter which does not break up readily after cooling.

Microstructures of Reduction Products

The development of microstructures in the sand grains during oxidation and reduction followed the patterns described by Edstrom (1953), Bitsianes and Joseph (1953). During oxidation runs, surface reaction zones of hema-

tite formed around the magnetite grains, and subsequently moved inwards until the grains were completely converted to hematite. The reverse action occurred during reduction, surface reaction zones of magnetite moving inwards until the grains were completely converted to magnetite.

Subsequently wustite appeared as fine filamentary networks throughout the magnetite grains, the filaments increasing in number and thickness until the grains consisted of wustite matrices containing residual islands and lamellae of magnetite and/or gangue. Initial metallisation started at this stage, metallic iron or iron carbide occurring as fine particles dispersed throughout the wustite matrices. These particles subsequently increased in size and number until the grains finally consisted of iron or iron carbide matrices containing gangue and residual iron oxides in the form of very finely dispersed particles and/or residual lamellae.

X-ray diffraction analysis confirmed that magnetite, wustite, and metallic iron (or iron carbide) were simultaneously present during advanced stages of reduction, and that magnetite persisted to a very advanced stage of metallisation. The typical microstructure of a final reduction product is shown in Fig. 10.

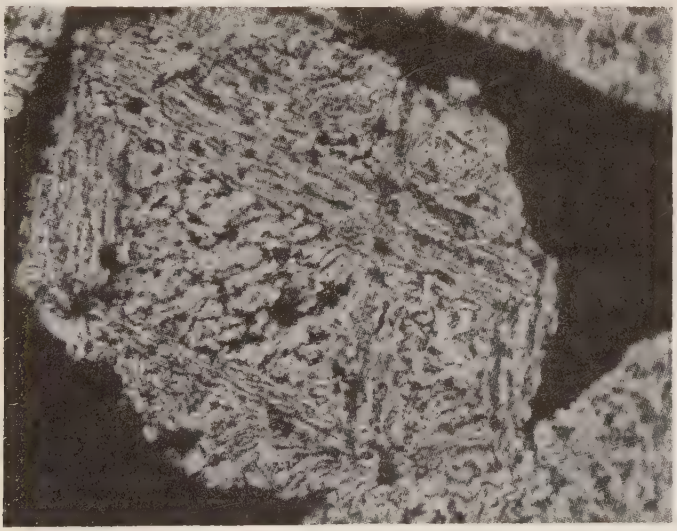

FIG. 10—Photomicrograph of reduced ironsand: Patea sand reduced with hydrogen for 3·3 hours at 900°C. × 560.

Deposited carbon, when present, formed rims around the metallised grains or filled interstices and cracks in the grains.

With preoxidised ironsands, individual grains behaved similarly during reduction, yielding a homogeneous product at any stage of reduction. On the other hand the natural unoxidised ironsands reduced in a heterogeneous manner, some of the grains becoming metallised with extreme rapidity while the majority of the constituent grains became metallised more slowly. This behaviour accounts for the very early appearance of metallic iron in the

reduction of unoxidised Patea ironsand with hydrogen at 750°C (see Fig. 6).

Microsections of samples taken during reduction runs on Taharoa ironsands revealed the presence of numerous locked particles of silicate gangue minerals which often partly enclosed the titanomagnetite grains. In such cases the gangue minerals were observed to retard the reduction of the enclosed iron oxides. The presence of these locked particles of gangue silicates probably accounts for the slightly lower reactivity of the Taharoa ironsand, as compared to the Patea ironsand.

DISCUSSION

Carbon Monoxide Reduction

Results obtained with carbon monoxide reduction of New Zealand ironsands at 600°C agree with those published by Stelling (1958) to the extent that preoxidation of the ore is necessary to obtain reasonable reactivity, and that sticking of the fluidized beds does not occur at this temperature. The gas utilisation with oxidised New Zealand ironsands is also comparable to that reported by Stelling (1958) if allowance is made for the difference in fluidization techniques. However, Stelling (1958) reported complete reduction to iron carbide, whereas only traces of carbide and metallic iron were formed in the reduction runs on oxidised Patea and Taharoa ironsand using carbon monoxide containing 10% of carbon dioxide. The purer Gillespie's Beach oxidised ironsand and the same reducing gas gave larger amounts of iron carbide. All of these runs were hampered by excessive carbon deposition which occurred in the latter stages. Results were improved by using purer carbon monoxide, containing 2% carbon dioxide, as the reducing gas, to the extent that oxidised Patea ironsand could be reduced in 8 hours at 600°C to a mixture of wustite, iron carbide and carbon which would react on heating above 700°C to form a nearly completely metallised product.

In conformity with Stelling's (1958) observations, sticking occurred in the reduction of oxidised Gillespie's Beach ironsand with carbon monoxide at 750°C. The oxidised Patea and Taharoa ironsands, however, show a higher possible operating temperature, up to 750°C, without sticking, but the reduction rates are relatively low.

The two titaniferous ironsands used, from Lake Taharoa and Patea, showed essentially similar behaviour in carbon monoxide reduction though the Taharoa material appeared to be slightly less reactive. This may have resulted from the slightly higher gangue content and larger proportion of locked particles in the Taharoa concentrate. Both of these ironsands exhibited a lower reactivity than the Gillespie's Beach material, which is in agreement with the findings of Specht and Zapffe (1946), that titania has an adverse effect on reduction rate.

Hydrogen Reduction

The runs on oxidised Gillespie's Beach material agree with published data (Squires and Johnson, 1957; Unterweiser, 1956; Kuzmick and Bruland, 1958) in that sticking occurred at all the temperatures tried. The H-iron process (Squires and Johnson, 1957) operates at a temperature below 600°C to avoid sticking.

With Patea ironsands, preoxidation was again found to increase the rate of reduction. The results obtained are in striking contrast to those obtained with the relatively pure Gillespie's Beach ore, in that sticking did not occur between 700° and 900°C. No explanation of the sticking at 600°C and again at 1,000°C can be offered, since the mechanism of sticking is not known. Reduction was very rapid at 900°C, on both the oxidised and unoxidised titaniferous ironsands. The reaction rates and gas utilisations compare very favourably with published data for the H-iron reduction process (Squires and Johnson, 1957).

CONCLUSIONS

1. Results obtained in these experiments show that preoxidation, which is easily effected in a continuously operated fluidized bed, accelerates the fluidized-bed reduction of New Zealand ironsands. This observation agrees with those of Stelling (1958) and Edstrom (1953).

The conditions for fluidized-bed reduction of titaniferous ironsands in these tests may be grouped in order of decreasing effectiveness as follows:

(a) Hydrogen at 900°C. (Highest reduction rates.)
(b) Hydrogen at 800° and 750°C, and the carbon monoxide hydrogen mixture at 900°C.
(c) Carbon monoxide at 600° and 750°C. (Lowest reduction rates.)

As indicated by Edstrom (1953) carbon dioxide in the carbon monoxide reducing gas seriously retards reduction at 600°C; a carbon dioxide content of not more than 2% appears desirable for effective reduction.

2. From comparison with experimental results on the relatively pure Gillespie's Beach ironsand, the gangue content of the titaniferous North Island ironsands is considered to be slightly detrimental in lowering the reactivity to carbon monoxide and hydrogen, and highly beneficial in extending the temperature ranges in which fluidized-bed reduction of these ores can be effected without sticking. The feasibility of fluidized-bed hydrogen reduction at 900°C and carbon monoxide reduction at 750°C is ascribed to the gangue content of these titaniferous ironsands.

3. In general the gas utilisations obtained in these experiments fall below the values theoretically attainable under equilibrium conditions at the operating temperatures (Edstrom, 1953). Since gas utilisation efficiency is affected by the method of operation of a fluidized-bed, higher reaction rates, and gas utilisations closer to the equilibrium values, could probably be attained by improved reactor design. In this respect a completely fluidized bed would be expected to operate more efficiently than the spouted bed used in these tests.

4. Sticking appears to be controlled by temperature, gas composition, and by the inherent properties of the materials fluidized. Reactor operation was found to have no major influence on bed sticking. Since reactor design would affect only the physical variables already studied in reactor operation (degree of agitation, gas velocities, etc.) the sticking tendencies in larger reactors, with either spouted beds or fully fluidized beds, would be expected to conform to the general pattern shown in the present tests.

ACKNOWLEDGMENTS

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CORROSION OF METALS IN WHANGAEHU RIVER AND RUAPEHU CRATER WATERS

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Summary

Corrosion tests and pH measurements have shown that, owing to their high acidities, the waters of the Whangaehu River at Tangiwai and the Ruapehu crater lake are highly corrosive to metals. Typical corrosion rate data are given for a number of alloys, of which only platinum and titanium were essentially immune to corrosion in these media. Other alloys such as zirconium, stainless steel, inconel, antimonial lead, and mild steel exhibited slight to extremely severe corrosion.

The pH of Whangaehu river water at Tangiwai varies seasonally from 2.6 to 7.1 according to the degree of contamination with acid water from the Ruapehu crater lake.

Monitoring of the river pH appears to be worthy of further investigation as a method of indicating crater-lake conditions potentially favourable for lahar flooding.

Introduction

During investigations of the Tangiwai railway disaster of 24 December 1953, caused by a lahar flood (N.Z. Tangiwai Railway Disaster, Board of Inquiry, 1954) in the Whangaehu River, the river water was noticed to be highly acid (pH as low as 2·6) and corrosive to metals. For example, a steel hand rail from the wrecked train, removed from the river after less than 12 weeks' immersion, showed severe general corrosion about $\frac{1}{16}$ inch deep.

On the recommendation of the Board of Inquiry into the Tangiwai Disaster, engineers of the New Zealand Railway Department decided to install an electric device to prevent recurrence of similar accidents by giving early warnings of floods. The warning system employs contacts mounted on a vertical concrete pylon in such a way that rising waters bridge successive contacts, closing electrical circuits to actuate remote alarm signals. Since permanence and reliability are essential in this device, the corrosive nature of the water had to be taken into account in designing the immersion electrodes. The corrosion investigations described below were undertaken to provide quantitative data on corrosion of metals for use in design of this equipment.

Water Characteristics

Periodic measurements of the pH of Whangaehu river water at Tangiwai indicated seasonal variations in the acidity of the water as shown in Fig. 1. These annual variations are consistent with Healy's observations (N.Z. Tangiwai Railway Disaster, Board of Inquiry, 1954) that acid water escapes from the crater lake on Mount Ruapehu via channels beneath the surrounding ice, and emerges to form a branch of the Whangaehu River.

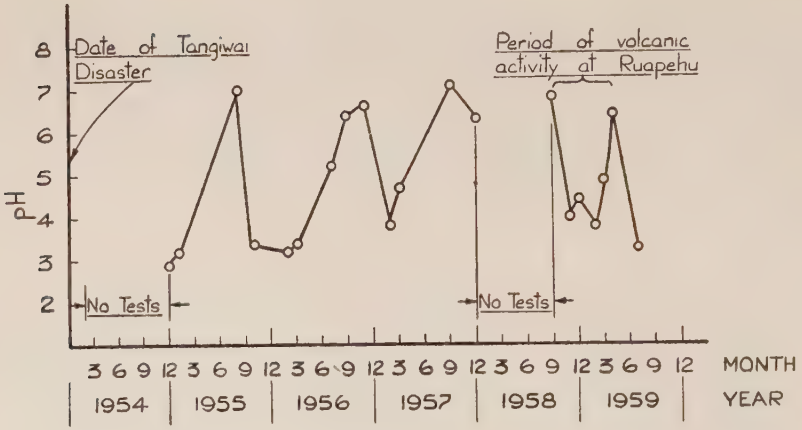

FIG. 1—Variations in pH of Whangaehu river water at Tangiwai

Presumably, in cold winter weather, sealing of the channels with ice may restrict or prevent the escape of crater water, causing the pH of the Whangaehu river water to rise to a near-neutral value of 6.6 to 7.1.

In warm summer weather, or in periods of volcanic activity at the crater, thawing of the ice apparently facilitates escape of crater water, which then mixes with the river water and lowers its pH. Confirmation of this theory is provided by typical analyses of the river and crater water given in Table 1 in which the chloride/sulphate ratios indicate that the mixing of fresh snow water with crater water would give compositions similar to that of Whangaehu river water, and by pH measurements taken at intervals up the Whangaehu River towards the crater of Mount Ruapehu. As shown in Table 2 the pH during typical summer periods decreases progressively from Tangiwai to the crater.

TABLE 1—Analyses of Whangaehu River and Ruapehu Crater Waters

	Whangaehu River Water			Ruapehu Crater Water†
Sample Date	9/3/56	12/9/56	4/2/59	10/1/59
pH	3·4	6·4	3·4	1·2
Chloride (Cl^-)*	89 ppm	30 ppm	142 ppm	3,480 ppm
Sulphate ($SO_4^=$)*	144 ppm	54 ppm	247 ppm	6,100 ppm
Fluoride (F^-)	7 ppm			
Total acidity to phenolphthalein (as ppm of $CaCO_3$)	102 ppm	2 ppm	202·5 ppm	11,300 ppm
Total acidity to methyl orange (as ppm of $CaCO_3$)	2 ppm	Nil	54 ppm	6,060 ppm
Ratio $Cl^-/SO_4^=$	0·62	0·56	0·58	0·57

*Accuracy claimed ± 2%.
†Crater water has a persistent odour of H_2S and probably contains H_2S and sulphur compounds not shown in these analyses.

TABLE 2—Variations in pH of Whangaehu River Water between Mt. Ruapehu and Tangiwai

Sampling Point	Sampling Date	pH
Ruapehu Crater, altitude 8,325 ft	7/7/1959	1·2
Whangaehu River, altitude 5,000 ft	22/4/1959	3·4
Whangaehu River, altitude 3,380 ft	22/4/1959	4·0
Whangaehu River, altitude 3,040 ft	22/4/1959	4·5
Whangaehu River, altitude 2,280 ft (at Tangiwai)	22/4/1959	6·4

From the corrosion viewpoint, the pH data are significant in indicating that the river water will be highly corrosive during the summer months when crater water escapes into the river, and much less corrosive during winter months when the outflow of crater water is restricted.

In addition, it was noticed that a strong smell of H_2S emanating from the Whangaehu river at Tangiwai persisted for more than a year after the railway disaster and subsequently disappeared. This may have resulted from the presence of sulphur and/or sulphide minerals in the muds washed down from the Ruapehu crater during the 1953 lahar flood, and their subsequent protracted reaction with the acid river water to evolve H_2S. Although no analytical confirmation of this transitory phenomenon can be offered, the presence of H_2S must be considered as a possible corrosion-promoting factor.

Alloys Tested

The compositions of alloys tested are given in Table 3. These alloys were selected for their potentially high resistance to corrosion by acid chloride waters, since it was already known from examination of Tangiwai wreckage that the common engineering alloys would exhibit very high rates of corrosion in Whangaehu river water.

Corrosion Test Methods

The following corrosion rate measurements were conducted by the standard A.S.T.M. coupon method (American Society for Testing Materials, 1946) with the usual precautions to avoid errors due to galvanic contact effects, and using electrolytic coupon cleaning methods recommended by Uhlig (1948):

A. Total immersion tests in the Whangaehu River at Tangiwai, conducted over the period 8/5/56 to 18/11/57, during which the pH of the river water varied from 3.6 to 7.1. This long exposure period was chosen to indicate average corrosion rates over periods of fluctuating pH.

B. Total immersion tests in the crater lake of Mount Ruapehu conducted from 10/1/59 to 31/1/59. These tests were conducted as a check on the relative corrosion resistance of the various alloys under conditions of the maximum severity that could possibly be encountered at or near Tangiwai during lahar floods.

TABLE 3—Compositions of Alloys Tested

Alloy	Per Cent Composition†																Remarks
	Ni	Cr	Mo	Fe	Si	Mn	Pb	Sb	Sn	As	Cu	Al	Ti	C	S	P	
Inconel	M	15·2		8·0	0·3	<0·1						0·05	0·2				
Corronel B	M	0·2	27·1	6·6	0·3	0·5					0·9	0·1		0·08			
Monel	67			1·4	<0·1	0·43					31	<0·05					
18/8/3 CrNiMo Stainless steel	7·9	17·6	3·16	M	0·3	0·29					0·3	0·005	<0·02	0·05		0·025	
High-silicon cast Iron				M	16·4	1·6								0·72			
Antimonial lead	<0·01			<0·01			M	9·9	0·02	0·01	0·03						
Aluminium				<0·005							<0·01	M					"Superpure" grade
Mild steel*	0·05	<0·1	<0·1	M	<0·1	0·3								0·06	0·06	0·06	Chain links
Titanium	Commercially pure																
Zirconium	Commercially pure																
Platinum	Commercially pure																

*Composition of mild steel chain links used for anchoring specimen assemblies.
†M = Major component.
< = less than.

C. A number of subsidiary field and laboratory corrosion tests to investigate aspects such as water-line attack, effects of water velocity, effects of stagnant conditions, presence of H_2S in the water, sensitivity of alloys to pitting attack etc.

Normal laboratory and field-testing methods (Champion, 1952) using Whangaehu river water, Ruapehu crater water, or mixtures thereof, were applied in these subsidiary investigations.

Test Results

Results of the total immersion tests are summarized in Table 4. In the subsidiary tests none of the alloys exhibited unusual sensitivity to water-line attack, and none of the alloys except zirconium showed abnormal acceleration of corrosion due to water velocities of the order attainable in river flow, or to stagnant water conditions. Localised pitting of zirconium occurred frequently in these subsidiary tests, particularly under stagnant water conditions at low pH.

TABLE 4—Average Corrosion Rates of Various Alloys in Whangaehu River and Ruapehu Crater Waters

Alloy	Average Corrosion Rates*, Corrosion Characteristics† in Brackets.	
	Whangaehu River Water, Total Immersion for 559 Days at Tangiwai	Ruapehu Crater Water, 50°C, Total Immersion for 21 Days
Platinum	<0·01 (Nil)	<0·1 (Nil)
Titanium	<0·01 (Nil)	<0·1 (Nil)
Zirconium	<0·01 (Nil)	<0·1 (M12, P1)
18/8/3 Cr Ni Mo stainless steel	<0·01 (Nil)	22 (Gu3, W22)
High-silicon cast iron	0·04 (Gu1)	8 (Gu3)
Inconel	0·2 (M71)	20 (Gu3, M71)
Aluminium	0·3 (M12, P1)	28 (Gu5)
Monel	0·2 (Ge2, N12)	14 (Gu3, M12)
Corronel B	1 (Gu3)	2 (Gu2)
Antimonial lead	2 (Gu3)	6 (Gu3, M71)
Mild steel‡	200 (Gu)	1,000 (Gu)

*Corrosion rates in mils per year, 1 mil = 0·001 inch.
†Corrosion characteristics according to the indexing system of Champion (1952).
‡Data for mild steel estimated from attack on chains and other steel attachments.

Deliberate additions of H_2S to mixtures of river and crater waters caused no significant changes in type or rate of corrosion of the alloys, except in the case of inconel, which exhibited accelerated corrosion and rapid pitting attack in the presence of H_2S.

DISCUSSION

The extremely aggressive corrosion characteristics of Ruapehu crater water and of Whangaehu river water contaminated with crater water are

clearly indicated by the corrosion rate data in Table 4, particularly the estimated rates of 1 inch per year, and 0.2 inch per year for mild steel.

The various alloys tested can be arranged in an order of merit for corrosion-resistant service in these media, as in Table 5, by consideration of their general surface filming properties. Platinum, whose corrosion resistance depends on nobility of the metal and not on the formation of protective surface films, showed virtual immunity to corrosion in all of the test media.

TABLE 5—Corrosion Resistance Ratings of Alloys Tested

	Group Alloys	Corrosion Resistance Rating	Remarks
(1)	Platinum Titanium	Excellent	No detectable attack in all test media.
(2)	Zirconium	Good	No detectable attack by Whangaehu river water. Local pitting in Ruapehu crater water.
(3)	18/8/3 CrNiMo stainless steel High-silicon cast iron Inconel Aluminium	Variable	No attack, local pitting, or slight general corrosion in Whangaehu river water. Moderate or severe general attack in Ruapehu crater water.
(4)	Monel Corronel B Antimonial lead	Moderate	Moderate or severe general corrosion in all test media.
(5)	Mild steel	Poor	Severe general corrosion in all test media.

The highly protective surface oxide films on titanium, to which the outstanding corrosion resistance of this metal is usually ascribed, were apparently sufficiently stable to withstand breakdown in all of the test media, so that titanium showed no detectable corrosion even in the highly aggressive crater water.

On the other hand the protective oxide films on zirconium were not sufficiently stable to afford complete protection in all test media. Consequently, only in the most aggressive media, e.g. crater water, did deep localized pitting occurr at a few weak points in the surface oxide films.

The third group in Table 5 also consists of alloys whose corrosion resistances depend primarily on the formation and maintenance of surface oxide films. In these cases again, the stability of the oxide films was not sufficient to afford complete protection in all media. Variable corrosion behaviour was exhibited, the alloys showing a tendency to local film breakdown with consequent pitting in the milder river water environment, and to complete film breakdown with resultant severe general corrosion in the more aggressive crater water.

Except in a few specific media, the alloys in the fourth group do not form highly protective surface films, and are not inherently noble alloys.

As a result, they exhibited moderate to severe general corrosion in all of the test media. Mild steel is not a noble metal and does not form highly protective oxide surface films. Its low resistance to corrosion in these highly acid media is therefore not surprising.

The cyclic variations in pH of the Whangaehu River (Fig. 1) are of further interest. Continuance of high river water pH for abnormally long periods, particularly periods extending into warm summer weather, would imply blockage or restriction of the crater outlet, accumulation of crater water behind a relatively unstable ice or rock barrier, possibly with increase in height of the lake surface due to thawing of surrounding snow. This would represent a potentially dangerous condition where a sudden outflow of crater water could be initiated by other factors such as warm rains, volcanic activity in the crater, earthquakes etc.

In this context it may be significant that the flood of December 1953 occurred after build-up of the crater lake level in summer months, and that two previous cold lahar floods from Ruapehu (floods of non-volcanic initiation; Feb. 1861, Jan. 1925; N.Z. Tangiwai Railway Distaster, Board of Inquiry, 1954) occurred in the late summer months.

This aspect of Whangaehu river pH appears to be worthy of further investigation as a possible method of indicating crater-lake conditions potentially favourable for lahar flooding.

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COLLECTION OF *HYLEMYIA SENECIELLA* (*MEADE*) (DIPTERA: MUSCIDAE) IN 1959 SEASON

By J. M. HOY, Entomology Division, Department of Scientific and Industrial Research, Palmerston North

(Received for publication 10 November 1959)

Summary

A total of approximately twenty thousand puparia of *Hylemyia seneciella* (Meade), the ragwort seed fly, were shipped to Australia during March 1959.

A flotation technique was used for recovery of puparia from sand trays in this season. The sieving technique used in the previous season did not result in a significant percentage of abnormal flies.

INTRODUCTION

In order to meet a request for further supplies of *H. seneciella* for shipment to Australia, four field collections of infested ragwort inflorescences were made during January and February 1959. The first 3 collections were made on 15 and 22 January and 5 February in the same area as that used for the 1958 collection (Hoy, 1958). In that season the bulk of the infested material was collected on the flatter portion of the 10 acre stand. In 1959 the level of infestation on that portion appeared to be lower than that encountered in 1958, and this may have been due to the amount of infested material removed in the previous season. The bulk of the ragwort in these 3 collections in 1959 was taken from the slopes behind the area used in 1958; here the level of infestation was similar to that observed on the flats in the previous season. In order to ensure a large number of puparia for shipment the area was again visited on 10–11 February when efforts were made to find further areas of ragwort in the same vicinity. An area of some 15 acres of ragwort was found within 500 yds of the first collecting area. This ragwort was growing in a damper situation and was much more heavily infested by seedfly than any observed previously. A large quantity of infested inflorescences was brought to the laboratory from this area on 11 February. The last 2 collections in 1959 were made 1 and 2 weeks later respectively than the last collection in 1958 and, as might have been expected, a large number of fully fed larvae dropped from the heads during transit. These larvae were transferred directly to the sand trays.

RECOVERY OF PUPARIA

Bundles of infested ragwort inflorescences were inverted over netting frames above sand trays in the insectary in the same manner as described by Hoy (1958). A total equivalent to 48 sand trays was used in the 1959 season, each tray with 4 bundles of ragwort. Because of the greatly increased quantity of plant material used in this season some paper-lined wooden boxes

were used as well as galvanised iron trays. These boxes were unsatisfactory as larvae tended to tunnel through the paper and pupate on the bottom of the box. Sand in trays and boxes was kept moist by occasional watering, care being taken to disturb the sand surface after each watering to allow larvae to penetrate.

Recovery of puparia from sand was commenced 14 days after the first trays were set up. Only a few larvae had failed to pupate within this period. Because of the possibility of damage to puparia recovered from sand by a sieving technique (Hoy, 1958) it was decided to use a flotation method in the current season. At that time no information was available from Australia on emergences of adult flies from puparia dispatched in 1958.

The coarser plant material which had dropped into the sand trays was scraped and blown from the surface. The top layer of sand containing the remaining plant debris and a few puparia was then placed in approximately 3–4 gallons of water in a large vertical-sided container. Puparia were recovered individually with a small plastic scoop and transferred to a large sheet of blotting paper to remove surplus moisture. The remaining sand in each tray was then treated in a similar manner but puparia were removed, as they floated on top of the water, with a 12 mesh to the linear inch sieve, gently washed in running water and transferred to the blotting paper. The remaining debris and a small number of puparia of the ragwort leaf-miner (*Phytomyza atricornis* Mg.) were removed from the blotter and puparia of *H. seneciella* were placed in washed sand in plastic holding dishes measuring $9\frac{1}{2}$ in. $\times$ $4\frac{3}{4}$ in. $\times$ 2 in. deep. Each dish held approximately 2,000 puparia.

Sand used in some of the earlier prepared collecting trays contained a proportion of silt and soil and this tended to form a scum on the surface of the water in the flotation container. Trays prepared later were made up with washed sand and these proved satisfactory.

Over the period 29 January to 27 February approximately twenty thousand puparia were recovered from the sand trays and stored in the small plastic dishes. The flotation technique was more time consuming than the sieving method described in an earlier paper (Hoy, 1958).

PACKING AND DISPATCH OF PUPARIA

Puparia were recovered from sand in the holding dishes by flotation. A 12 mesh screen was used to collect puparia from the water surface and they were again transferred to a large sheet of blotting paper to remove excess moisture. To facilitate handling of the consignment on arrival at Canberra, puparia were packed in layers in lightly moistened vermiculite in a wooden container, The consignment left Palmerston North by express air freight on 3 March and was received in Canberra on 4 March.

EMERGENCE OF ADULT FLIES FROM 1958 CONSIGNMENT

Dr G. F. Bornemissza, Division of Entomology, Commonwealth Scientific and Industrial Research Organisation, Canberra, has supplied the following information (pers. comm.) on emergence of flies from the 1958 consignment of *H. seneciella*. A total of 2,873 flies emerged from approximately 4,000

puparia sent from New Zealand. During the earlier part of the season flies that emerged were normal and vigorous. During this period about 20 crippled flies were encountered but this would be the usual proportion expected and could not be attributed to the sieving technique. Later in the season a heat wave, with daily temperatures varying between 105° and 113°F, was experienced at the field quarantine station in Gippsland. Owing to lack of adequate means of temperature control at the field station, flies emerging during this period died even before stretching their wings.

REFERENCE

HOY, J. M. 1958: The Collection of *Hylemyia seneciella* (Meade) (Diptera, Muscidae) for Shipment to Australia. *N.Z. J. Sci. 1*: 417–22.

INFLUENCE OF POPULATION DENSITY ON THE ADRENAL AND BODY WEIGHTS OF THE WILD RABBIT *Oryctolagus cuniculus* L. IN NEW ZEALAND

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Summary

A total of 523 European wild rabbits from 4 controlled populations ranging from 1 rabbit to 5 acres up to 23 rabbits to the acre, in the North and South Islands of New Zealand, were examined; their sex, age, body and adrenal weights, and the amount of fat were recorded.

The season of collection did not affect the body weight. Rabbits from Gore, in the South Island, were, however, significantly heavier than those collected from the Wairarapa, in the North Island, at the same season and from populations of similar density. There was no significant difference in fat deposits between these two areas, but in medium and high populations young rabbits had almost as much fat as adult rabbits.

Contrary to what obtains for other mammals, male wild rabbits in New Zealand, like American cottontail rabbits, have relatively heavier adrenals than have females; this characteristic may be peculiar to Lagomorphs.

A direct relationship between adrenal weights and population density is demonstrated, but is much less pronounced than in other mammals studied so far.

It is shown that with increasing population density the condition, as evinced by fat content, improved. Contrary to what is known of other mammalian species, such as rats or mice, crowding results in an improvement of the condition of the rabbit, especially of the young. It is suggested that a fairly high population density is essential for the welfare of the rabbit.

Introduction

Several authors have suggested that changes in population density exert important effects on the physiology of individuals in populations of small mammals. Chitty (1952 and 1955) and Clarke (1953) postulated that "intraspecific strife is of primary importance in determining the population size in the vole *Microtus agrestis*." Christian (1950) suggested that, by inducing stress in the members of a population, social competition is related to population density and evokes various physiological responses to stress, especially of the endocrines. Of these the most outstanding is an enlargement of the adrenal cortex with histological signs of hyperactivity reflecting changes in the amount of cortical hormones produced (Selye, 1950). Thus the amount of cortical tissue, as measured by the weight of adrenals is an indirect indication of the degree of stress. Christian (1959) reviewed experiments which were conducted with the object of exploring, in populations of small mammals, the relationships between population density and endocrine responses; he concluded that all these populations have "a behavioral-endocrine feed-back system which can and does regulate their growth by its effect on reproduction and unquestionably on adult mortality." An intensive study of the population dynamics of the wild

rabbit (*Oryctolagus cuniculus* L.), carried out by the Animal Ecology Section, provided an opportunity to find out whether the weight of the adrenals, the body weight, and the condition were related to population density.

MATERIAL AND METHODS

Ideally, the material for this study should have been collected from natural populations, situated in the same district and sampled at the same time. The reduction over the last decade of rabbit populations throughout New Zealand, however, prevented fulfilment of the above requirements. The samples were therefore obtained from three districts and their collection extended over a period of nine months.

A total of 523 rabbits from 4 populations of different densities subsequently described as sparse, low, medium and high were used. The approximate population densities were estimated either from sight counts by one of the authors (K.W.), or from the results of control measures carried out at the time of obtaining rabbits for this project.

These four populations may be briefly described as follows:

Sparse: Population density was estimated at about 1 rabbit to 5 acres. This sample of 134 rabbits was obtained by night shooting, from the Wairarapa district, North Island, in June 1957 (41 rabbits), and in October 1957 (43 rabbits), and from the Gore district, South Island, in October 1957 (50 rabbits). In each of these two districts the rabbits came from several localities a few miles apart, and have been pooled.

Low: Density about 1 rabbit to 1 acre. This sample of 181 rabbits was obtained by strychnine poisoning in February 1958 at Awatea Station, Hawke's Bay. This area, in the partly scrub-covered hills about 15 miles east of Hastings, some years ago supported a medium to high rabbit population.

Medium: Density about 3 rabbits to 1 acre. This sample of 111 rabbits was obtained in May 1957 (93 rabbits) and July 1957 (18 rabbits), by trapping at Kourarau Station, Wairarapa. This is a few miles from the localities where the sparse population sample (see above) was collected; up to two years ago this property supported a heavy rabbit population.

High: Density about 23 rabbits to 1 acre. This sample of 97 rabbits was obtained in August 1957 from Waikoikoi, near Gore, Southland, by poisoning with 1081.

All these populations, with the exception of Waikoikoi, had been subjected to regular control measures of various kinds and effectiveness. At Waikoikoi no control measures had been applied for several months prior to the kill in August 1957.

In the laboratory the rabbits were dissected and data on sex, paunched weight (Watson and Williams, 1955), fat content, adrenal weight, and age were obtained. The adrenals were cleaned from adherent fat and weighed to the nearest 0·005 g. The condition of the rabbits was assessed from the amount of fat in the body cavity round the kidneys, and the

amount of fat was recorded as follows: 0 – no fat; 1 – little fat in body cavity; 2 – intermediate, substantial amount of fat but kidneys not covered; 3 – large amount of fat, completely covering kidneys.

Although 523 rabbits were collected, only 493 were used for the analysis, because the adrenals of some were damaged by shot. Finally, all rabbits were divided into three age groups, using methods of age determination based on the epiphyseal fusion of the tibia (Watson and Tyndale-Biscoe, 1953) and the lumbar vertebrae (Taylor 1959); the groups are (i) less than 10 months, (ii) 10–33 months, and (iii) more than 33 months.

RESULTS

Characteristics of Populations Examined

SEX RATIO

Table 1 shows the number of males and females and the sex ratio of each population. The proportion of males in the sparse, low, and high populations was 48%; the higher proportion in the medium population is probably attributable to the method of collection, as for this sample traps were set in the open (Watson, 1957).

TABLE 1—Sex Ratio

Population Density	Number in Sample			
	Males	Females	Total	% Males
Sparse	64	70	134	47·8
Low	87	94	181	48·1
Medium	68	43	111	61·3
High	47	50	97	48·4
Total	266	257	523	50·9

AGE COMPOSITION

Table 2 gives the numbers of each age group for each population. The proportions of the three age groups varied from population to population, the differences being significant at the 0·1% level for both males and females, the proportions being affected by the season of collection and also by the different intensity of control measures. Thus the large proportion of young rabbits under 10 months in the low and medium populations is due to the rabbits being killed soon after the end of the main breeding season (Watson, 1957). Conversely, the collection of the high population was made after most of the previous season's young rabbits had entered the 10–33 months age group.

TABLE 2—Age Composition

Population Density	Males				Females				Grand Total
	<10 Mths	10–33 Mths	>33 Mths	Total	<10 Mths	10–33 Mths	>33 Mths	Total	
Sparse ..	10	16	5	31	9	26	3	38	69
	3	11	11	25	2	8	8	18	43
Low ..	40	35	12	87	35	40	16	91	178
Medium ..	40	18	6	64	21	16	5	42	106
High ..	6	36	5	47	7	33	10	50	97
Total ..	99	116	39	254	74	123	42	239	493

NOTE: The upper and lower figures for the sparse population represent samples taken in Wairarapa, North Island, and Gore, South Island, respectively.

CONDITION

(a) *Paunched Weight*

(i) POPULATION DENSITY: Table 3 shows the condition of rabbits as measured by paunched weight, for the 4 populations. The most noticeable feature about this characteristic is the increase in the paunched weights of the young rabbits, both male and female, as the population becomes more dense. In the sparse population, both the male and the female less than 10 months are about 300 g lighter than in the 10–33 months age group, while in the high population there is practically no difference. The increase in the less than 10 months group over the 4 populations is significant at 0·1% for the males and at 5% for the females; the differences in the 10–33 months group are significant at the 1% level for both males and females; and for the over 33 months group there is a significant difference in the females at the 5% level but none in the males.

The date of the collection of the various samples provides a partial explanation of these findings. The sparse population was collected at the beginning of the breeding season when a few young animals (3 males and 3 females) were found in the sample; the high population was killed just before the beginning of the breeding season, when most of the young rabbits from the previous breeding season had moved into the next age group.

(ii) SEASONAL DIFFERENCE: Table 4 compares the paunched weights of two samples from sparse populations in the Wairarapa, collected in June and October 1957. With the exception of those for young females, the mean weights of all groups including totals show no significant difference between June and October. It would appear that the seasonal effect as measured by these two samples did not affect the paunched weight to any large extent.

(iii) LOCALITY DIFFERENCE: Table 5 compares the paunched weights of rabbits from sparse populations in the Wairarapa and near Gore, both collected in October 1957. For all three age groups, rabbits from Gore are heavier than those from populations of similar density in the Wairarapa.

TABLE 3—Mean Paunched Weights (in grams)

Population Density	No. of Rabbits	<10 Months	10–33 Months	>33 Months	Total
		MALES			
Sparse ..	56	1,117 (±38)	1,391 (±26)	1,497 (±36)	1,342 (±18)
Low ..	87	1,206 (±22)	1,483 (±23)	1,492 (±40)	1,357 (±15)
Medium ..	64	1,245 (±22)	1,376 (±32)	1,450 (±56)	1,299 (±17)
High ..	47	1,417 (±56)	1,397 (±23)	1,490 (±61)	1,410 (±20)
Total ..	254	..	..	..	1,348 (± 9)
		FEMALES			
Sparse ..	56	1,100 (±42)	1,390 (±24)	1,515 (±42)	1,366 (±18)
Low ..	91	1,143 (±23)	1,420 (±22)	1,506 (±34)	1,329 (±14)
Medium ..	42	1,211 (±30)	1,300 (±34)	1,350 (±62)	1,260 (±21)
High ..	50	1,329 (±52)	1,398 (±24)	1,495 (±44)	1,408 (±20)
Total ..	239	..	..	..	1,343 (± 9)

The figure given in brackets after each mean is the Standard Error (S.E.) for that mean, this being calculated by dividing the S.E. for an individual rabbit by the square root of the number of rabbits in that group (the S.E. for the males and females have been calculated separately). Thus we have assumed that the variance is constant within each of the two sex groups. If we wish to make a test of significance between any two of the means then the "t" test, using these S.E.s, will be sufficient. The method is as follows:

(1) Square the S.E.s of each of the two means.

(2) Sum these two squares.

(3) Take the square root of this sum.

(4) Divide the difference between the two means, by this square root, which gives us a "t" value.

(5) The significance of the difference between the two means is then found by comparing this "t" value, with the corresponding value in a table of "t", for 250 degrees of freedom, e.g., at the 5% level this is approximately 1·97. The same procedure can be used with the other tables.

TABLE 4—Paunched Weights in the Wairarapa in Different Seasons

Date	No. of Rabbits	<10 Months	10–33 Months	>33 Months	Total
Males—					
June ..	23	1,083 (±46)	1,371 (±40)	1,475 (±97)	1,267 (±29)
October ..	13	1,050 (±69)	1,308 (±56)	1,367 (±79)	1,242 (±38)
Females—					
June ..	18	1,171 (±52)	1,371 (±52)	1,450 (±69)	1,311 (±33)
October ..	30	813 (±69)	1,368 (±29)	1,488 (±69)	1,310 (±25)

Although only three of the six groups are significant, viz. less than 10 months males, 10–33 months and over 10 months females, a comparison of the total mean weights of rabbits from these two localities shows a very highly significant difference (0·1% level).

TABLE 5—Paunched Weights for Sparse Density in Different Localities

Locality	No. of Rabbits	<10 Months	10–33 Months	>33 Months	Total
Males—					
Gore ..	28	1,230 (±61)	1,459 (±41)	1,533 (±40)	1,450 (±26)
Wairarapa ..	13	1,050 (±69)	1,308 (±56)	1,367 (±79)	1,242 (±38)
Females—					
Gore ..	21	1,425 (±98)	1,450 (±44)	1,556 (±46)	1,493 (±30)
Wairarapa ..	30	813 (±69)	1,368 (±29)	1,488 (±69)	1,310 (±25)

It is obvious from Table 5 that localities contribute largely to the difference in paunched weight. The reduction of rabbit populations in New Zealand by recent control measures made it difficult to duplicate the four density groups in each island, although it was found possible to

TABLE 6—Mean Paunched Weight by Islands

Population Density	No. of Rabbits	<10 Months	10–33 Months	>33 Months	Total
		NORTH ISLAND			
Males—					
Sparse ..	31	1,020 (±43)	1,359 (±34)	1,410 (±61)	1,258 (±25)
Low ..	87	1,206	1,483	1,492	1,357
Medium ..	64	1,245	1,376	1,450	1,299
Total ..	182	..	..	..	1,321 (±10)
Females—					
Sparse ..	38	1,067 (±42)	1,375 (±26)	1,483 (±80)	1,311 (±22)
Low ..	91	1,143	1,420	1,506	1,329
Medium ..	42	1,211	1,300	1,350	1,260
Total ..	171	..	..	..	1,308 (±11)
		SOUTH ISLAND			
Males—					
Sparse ..	25	1,200 (±79)	1,459 (±41)	1,541 (±41)	1,464 (±27)
High ..	47	1,417	1,397	1,490	1,410
Total ..	72	..	..	..	1,428 (±16)
Females—					
Sparse ..	18	1,425 (±98)	1,431 (±49)	1,575 (±49)	1,494 (±33)
High ..	50	1,329	1,398	1,495	1,408
Total ..	68	..	..	..	1,431 (±17)

(The means and S.E.'s for low, medium, and high are the same as in Table 3.)

duplicate the sparse density. In statistical language this inability to duplicate is known as "confounding" – the difference in density is confounded with difference in place and we are not able to separate these two differences. We were unable to find low or medium populations in the South Island, or a high population in the North Island.

Table 3 showed that there was an increase in the paunched weight of young rabbits with the increase of population density; and while Table 4 showed that there was no significant difference attributable to the season, Table 5 shows a significant difference attributable to locality, at least in the sparse population. In addition to these, it is possible that there are other differences between the two islands, owing to climatic or genetic factors. In Table 6, therefore, paunched weights have been broken down into North Island and South Island.

It is to be noted that the same trend of increasing weight in young rabbits with increasing density, as was shown in Table 3, is maintained in the North Island. It is difficult to make any valid assessment for the South Island because the number of young rabbits was too small (3 males and 2 females) in the sparse population.

(b) *Fat*

(i) POPULATION DENSITY: Table 7 shows that there is, on the whole, no difference in fat deposition attributable to age. Populations of different density are, however, noticeably different; there was relatively low fat content in the sparse and low populations, the content increased in the medium, and was very high in the high population. The differences between the four populations, over all age groups, were significant at the 0·1% level both for males and for females.

TABLE 7—Index of Mean Fat Content

Population Density	No. of Rabbits	<10 Months	10–33 Months	>33 Months	Total
Males—					
Sparse ..	56	1·1 (±0·17)	1·5 (±0·12)	1·7 (±0·16)	1·4 (±0·08)
Low ..	87	1·0 (±0·10)	1·2 (±0·11)	1·3 (±0·18)	1·1 (±0·07)
Medium ..	64	1·5 (±0·10)	1·7 (±0·15)	1·5 (±0·26)	1·6 (±0·08)
High ..	47	2·2 (±0·26)	2·1 (±0·10)	2·4 (±0·28)	2·2 (±0·09)
Total ..	254	..	..	..	1·5 (±0·04)
Females—					
Sparse ..	56	1·2 (±0·22)	1·4 (±0·13)	1·5 (±0·22)	1·4 (±0·10)
Low ..	91	1·2 (±0·13)	1·5 (±0·12)	1·1 (±0·19)	1·3 (±0·08)
Medium ..	42	2·0 (±0·16)	2·4 (±0·19)	1·0 (±0·33)	2·1 (±0·11)
High ..	50	2·9 (±0·28)	2·7 (±0·13)	2·9 (±0·23)	2·8 (±0·10)
Total ..	239	..	..	..	1·8 (±0·05)

Measured by scores 0, 1, 2, or 3. (See p. 105).

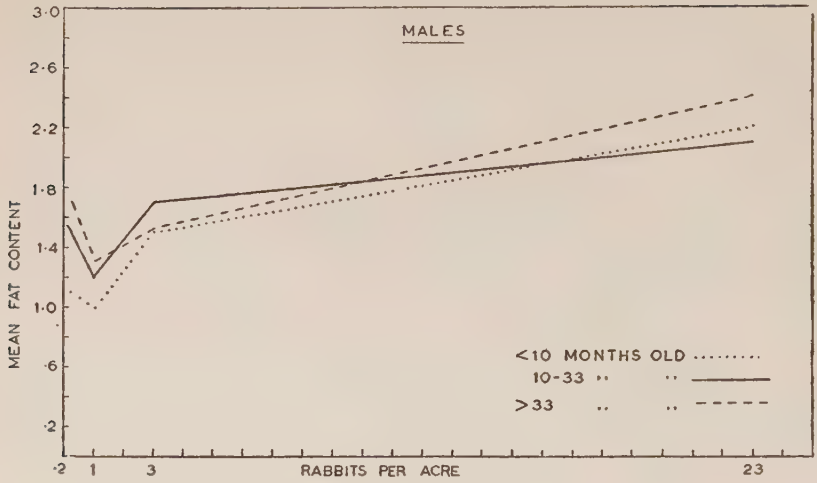

FIG. 1—Mean fat content plotted against population density (males).

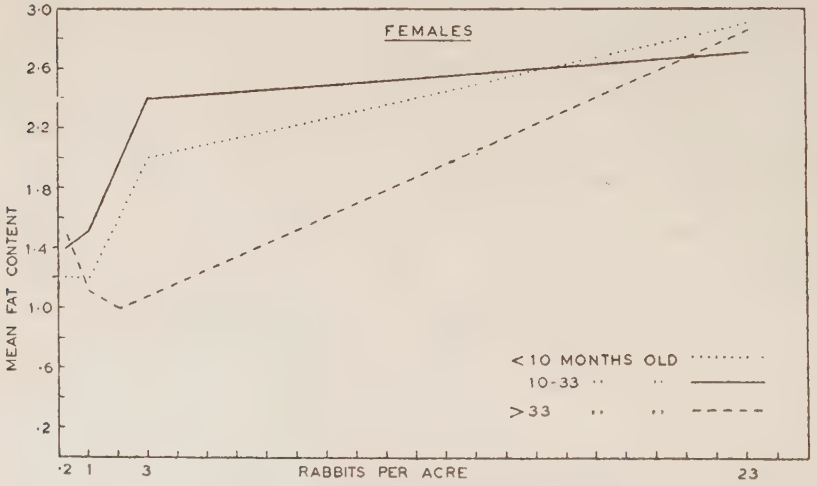

FIG. 2—Mean fat content plotted against population density (females).

Table 7, and Figs. 1 and 2, show that the increase in mean fat content does not correspond linearly to increase in population density. However, although Figs. 1 and 2 show a general increase from sparse to high population, there is a dip, marked in the case of the males, in the low population. There seems to be no evidence which would provide an adequate explanation for this dip. Even though the density is partly confounded with place, as stated above, it is legitimate to test the difference between the two islands in the case of sparse population as we have done with the paunched weights (Table 6).

There was no significant difference between the sparse populations at Gore and the Wairarapa in the mean fat content of either male or female rabbits. In Figs 1 and 2 the populations from all localities have been amalgamated. Although such amalgamation might not be fully justified, the fact that no significant locality difference in the sparse populations was found, lends some confidence in presenting the results in this manner.

We note that as was shown previously for paunched weights, young rabbits of both sexes, from sparse and low populations, have less fat than the adults; in the medium and high populations however, young rabbits have almost as much fat as the adults.

(ii) LOCALITY DIFFERENCE: Table 8 records the mean fat content by the North and South Islands, separately.

TABLE 8—Index of Mean Fat Content by Islands

Population Density	No. of Rabbits	<10 Months	10–33 Months	>33 Months	Total
		NORTH ISLAND			
Males—					
Sparse ..	31	1·2 (±0·20)	1·5 (±0·16)	1·8 (±0·28)	1·5 (±0·11)
Low ..	87	1·0	1·2	1·3	1·1
Medium ..	64	1·5	1·7	1·5	1·6
Total ..	182	..	..	..	1·3 (±0·05)
Females—					
Sparse ..	38	1·1 (±0·25)	1·6 (±0·15)	2·0 (±0·43)	1·5 (±0·12)
Low ..	91	1·2	1·5	1·1	1·3
Medium ..	42	2·0	2·4	1·0	2·1
Total ..	171	..	..	..	1·5 (±0·06)
		SOUTH ISLAND			
Males—					
Sparse ..	25	1·0 (±0·36)	1·4 (±0·19)	1·7 (±0·19)	1·4 (±0·13)
High ..	47	2·1	2·1	2·4	2·2
Total ..	72	..	..	..	1·9 (±0·07)
Females—					
Sparse ..	18	1·0 (±0·53)	1·5 (±0·26)	0·9 (±0·26)	1·3 (±0·18)
High ..	50	2·9	2·7	2·9	2·8
Total ..	68	..	..	..	2·3 (±0·09)

The means and S.E.'s for low, medium, and high are the same as in Table 4.

It can be seen that a similar picture of fat deposition increasing with population density is also obtained when the material is split by islands.

Adrenal Weight

INTRODUCTORY

The adrenals of the rabbit lie usually in a large quantity of fat about equidistant from the opening of the renal vein into the vena cava inferior and the kidney on the same side. The adrenals are oval, reniform or elongated in shape and lie with their longest diameter parallel to the axis of the body. Brown *et al* (1926) examined 644 domestic rabbits of both sexes. These had a mean paunched weight of 1841 g and a mean adrenal weight of 0·3833 g. According to Bachmann (1954) the weight of adrenals in immature mammals is approximately the same in both sexes; but adrenals of mature females are as a rule heavier than those of males. Christian (1953) found that the cottontail rabbit (*Sylvilagus floridanus* Gray) is an exception to this rule.

LEFT-RIGHT DIFFERENCE

As in many other mammals the left adrenal of the rabbit is generally heavier than the right. Table 9 shows that this difference was significant for almost all populations and age groups and that the mean difference between left and right adrenals is 0·02 g both for males and for females.

TABLE 9—Mean Weights of Left and Right Adrenals

Population Density	<10 Months		10–33 Months		>33 Months		Totals	
	L.	R.	L.	R.	L.	R.	L.	R.
Males—								
Sparse	0·17	0·10	0·18	0·17	0·18	0·16	0·18	0·15
Low	0·08	0·07	0·16	0·15	0·20	0·20	0·13	0·12
Medium	0·15	0·13	0·18	0·17	0·18	0·16	0·16	0·14
High	0·18	0·18	0·23	0·21	0·26	0·19	0·23	0·21
Total	..	..	..	..	..	..	0·17	0·15
Females—								
Sparse	0·15	0·12	0·16	0·12	0·17	0·13	0·16	0·12
Low	0·08	0·07	0·10	0·09	0·10	0·08	0·09	0·08
Medium	0·13	0·11	0·13	0·10	0·14	0·13	0·13	0·11
High	0·16	0·15	0·15	0·13	0·17	0·14	0·15	0·13
Total	..	..	..	..	..	..	0·13	0·11

SEX DIMORPHISM

From Table 10 it can be seen that the adrenals of males over all age groups are much bigger than those of females, being generally about 40% heavier; in this the European rabbit is similar to the cottontail.

TABLE 10—Mean Adrenal Weights of Males and Females

Population Density	Males	Females	Increase of Males over Females
Sparse	0·327	0·258	27
Low	0·248	0·178	39
Medium	0·310	0·230	35
High	0·436	0·287	52
Total	0·316	0·229	38

ADRENAL WEIGHT AS FUNCTION OF BODY WEIGHT

Table 11 shows that the correlation coefficients between paunched and adrenal weights are generally small, some being in fact negative, and only two being significant (at the 5% level). Thus there is generally very little correlation between these two characteristics in the New Zealand wild rabbit.

TABLE 11—Correlations between Paunched Weight and Adrenal Weight

Population Density	Males			Females		
	<10 Months	10–33 Months	>33 Months	<10 Months	10–33 Months	>33 Months
Sparse	0·23	0·49	— 0·12	0·81*	0·05	— 0·48
Low	0·23	— 0·04	0·18	0·26	0·19	— 0·07
Medium	0·27	0·47*	0·50	0·21	0·09	0·18
High	— 0·36	0·01	— 0·38	— 0·38	0·00	0·48

*Significant at the 5% level.

At this stage it is interesting to compare the adrenal weight as a percentage of body weight of wild rabbits in New Zealand with the cottontail and with the domestic rabbits. In the data on domestic rabbits published by Brown *et al.* (1926) no differentiation was made between age and sex. Christian (*in litt.*) recorded the sex, dead weight, and the weight of adrenals in 37 and 26 cottontail rabbits collected in May and October 1952 respectively, from a population on Parson's Island, Maryland, estimated at about ten rabbits to an acre. On the assumption that the relationship between dead and paunched weight in the cottontail is similar to that of the wild rabbits in New Zealand (Watson and Williams, 1955), Table 12 compares adrenals as percentage of paunched weight of the cottontail, the domestic, and the New Zealand wild rabbit.

TABLE 12—Adrenals as Percentage of Paunched Weight
(No. of rabbits in parentheses)

	Cottontail	Domestic	European Wild Rabbits in New Zealand
Males	0·0326 (31)	..	0·0234 (254)
Females	0·0202 (32)	..	0·0171 (239)
Total	0·0261 (63)	0·0208 (644)	0·0203 (493)

It may be seen from Table 12 that there is little difference between domestic and New Zealand wild rabbits. However, as Brown did not differentiate between males and females in his sample, and as these are known to be widely different this conclusion is tentative. On the other hand it appears that the cottontail rabbit has a relative adrenal weight about 30% heavier than the others.

ADRENALS AS FUNCTION OF FAT CONTENT

Table 13 shows the correlations between fat content and adrenal weight.

TABLE 13—Correlation Coefficients between Fat Content and Adrenal Weight

Population Density	Males			Females		
	<10 Months	10–33 Months	>33 Months	<10 Months	10–33 Months	>33 Months
Sparse	0·24	0·10	0·57*	0·44	0·03	0·26
Low	0·01	−0·06	0·46	0·01	−0·23	0·01
Medium	−0·07	0·08	0·36	−0·04	0·02	**
High	−0·07	−0·13	−0·25	−0·81*	−0·11	−0·02

*Significance at the 5% level.
**The number of rabbits in this group was too small to give a reliable figure.

Although it is true that only two of these coefficients are significant, nevertheless it is worthwhile noting that whereas all the coefficients in the sparse group are positive, all in the high group are negative. This shows that there is a slight but consistent trend within each of these two populations. Within the sparse group there is a tendency for the fat content to increase as the adrenal weight increases, while in the high group the fat content decreases as the adrenal weight increases.

POPULATION DENSITY

Table 14 compares the mean weights of adrenals in populations of varying densities.

TABLE 14—Mean Adrenal Weight

Population Density	No. of Rabbits	<10 Months	10–33 Months	>33 Months	Total
Males—					
Sparse ..	56	0·264 (±0·051)	0·346 (±0·035)	0·346 (±0·046)	0·327 (±0·024)
Low ..	87	0·157 (±0·029)	0·300 (±0·031)	0·401 (±0·053)	0·248 (±0·020)
Medium	64	0·283 (±0·029)	0·359 (±0·043)	0·342 (±0·075)	0·310 (±0·023)
High	47	0·368 (±0·075)	0·446 (±0·031)	0·448 (±0·082)	0·436 (±0·027)
Total	254	..	..	..	0·316 (±0·011)
Females—					
Sparse	56	0·245 (±0·023)	0·261 (±0·013)	0·260 (±0·023)	0·258 (±0·010)
Low ..	91	0·158 (±0·013)	0·195 (±0·012)	0·179 (±0·019)	0·178 (±0·008)
Medium	42	0·220 (±0·016)	0·230 (±0·019)	0·276 (±0·033)	0·230 (±0·012)
High ..	50	0·306 (±0·028)	0·277 (±0·013)	0·308 (±0·024)	0·287 (±0·011)
Total	239	..	..	..	0·229 (±0·005)

In the males, significant differences in mean adrenal weights between populations were found in the under 10 months group (0·1%) and in the 10 to 33 months group (5%), but in the over 33 months group the difference was not significant; while in the females there were significant differences at the 0·1% level in each of the age groups. Also noticeable is the fact that the young rabbits tend to have smaller adrenals than the adults. Also, with the exception of young rabbits in the low population, males have heavier adrenals than the females. In most cases these differences are significant.

Table 14 and Figs 3 and 4 show that the adrenal weight increases generally from sparse to high, with a dip in the low population; as with fat content, we are unable to offer any explanation for this dip. Old males over 33 months provide an exception to the dip although the same general trend can be observed.

LOCALITY DIFFERENCE

A comparison of adrenal weight by North and South islands and according to the density of the population is given in Table 15. The increase from sparse to high is observable in the South Island but there is no such increase in the North Island.

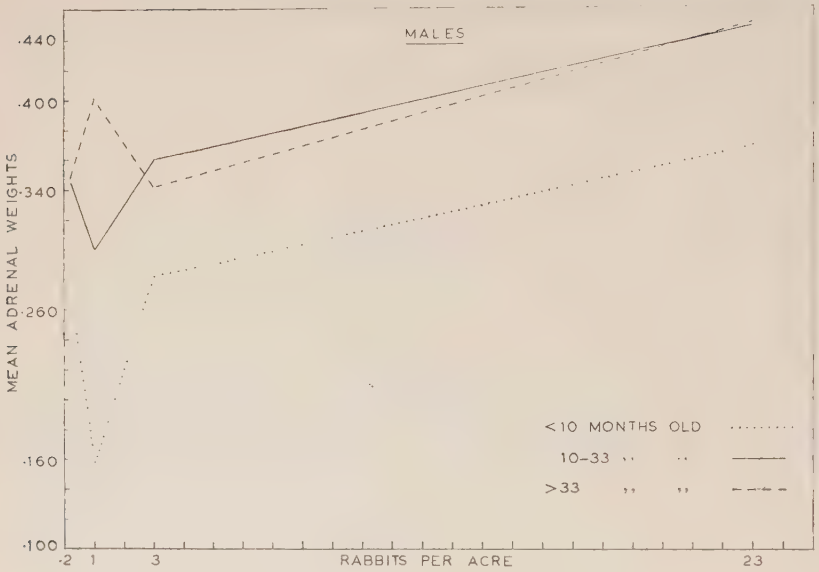

FIG. 3—Mean adrenal weight plotted against population density (males).

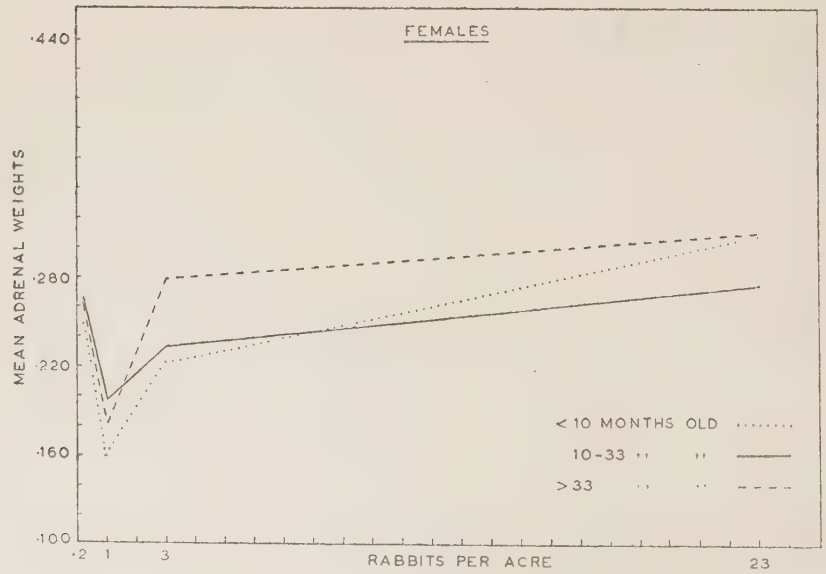

FIG. 4—Mean adrenal weight plotted against population density (females).

TABLE 15—Mean Adrenal Weight by Islands

Population Density	No. of Rabbits	<10 Months	10–33 Months	>33 Months	Totals
		NORTH ISLAND			
Males—					
Sparse	31	0·283 (±0·058)	0·298 (±0·046)	0·384 (±0·082)	0·307 (±0·033)
Low ..	87	0·157	0·300	0·401	0·248
Medium	64	0·283	0·359	0·342	0·310
Total	182	..	..	..	0·280 (±0·014)
Females—					
Sparse	38	0·241 (±0·025)	0·264 (±0·015)	0·300 (±0·043)	0·262 (±0·012)
Low ..	91	0·158	0·195	0·179	0·178
Medium	42	0·220	0·230	0·276	0·230
Total	171	..	..	..	0·212 (±0·006)
		SOUTH ISLAND			
Males—					
Sparse ..	25	0·203 (±0·106)	0·415 (±0·055)	0·328 (±0·055)	0·327 (±0·036)
High ..	47	0·368	0·466	0·448	0·436
Total	72	..	..	..	0·406 (±0·020)
Females—					
Sparse	18	0·260 (±0·053)	0·235 (±0·026)	0·241 (±0·026)	0·241 (±0·018)
High ..	50	0·306	0·277	0·308	0·287
Total	68	..	..	..	0·275 (±0·009)

The means and S.E.'s for low, medium, and high are the same as in Table 14.

The differences between the two islands were tested, for the sparse population (see pp 108–9) and no significant difference was found for either male or female. Consequently, as with mean fat content, we have amalgamated the populations in Figs 3 and 4.

DISCUSSION

Difference in Size of Rabbits

With one exception the sex ratio was similar in all samples but there were differences in age structure partly due to the intensity of control methods and partly to the date of collection. The paunched weights of rabbits collected from the same locality did not vary significantly between winter and spring for both males and females. On the other hand a highly significant locality difference was found between paunched weights of rabbits from sparse populations in the Wairarapa (41°S), North Island, and

Gore (46°S), South Island, collected at approximately the same date—the rabbits from Gore being much heavier. Though only 475 miles apart these two localities produced a significant difference in weight at the 1% level, of 208 g for males and 183 g for females. No other comparable material is available and it would be interesting to follow up this lead by examining rabbits collected at the same time from a similar altitude, but from varying latitudes in both islands.

Weight of Adrenals of Wild Rabbits

With the exception of rats, surprisingly few data are available on adrenal weights of animals of known age and from populations of known density. In the European wild rabbit in New Zealand as in its American relative, the cottontail (Christian 1953), the adrenals of mature males are heavier than those of mature females. All other species of mammals for which adequate data are available display the opposite characteristic; possibly this feature is common to Lagomorphs. On the other hand, wild rabbits like all other mammals studied (Bachmann 1954), have a significantly larger left adrenal.

Christian (1953) demonstrated that adrenal weight is a function of body weight; in mammals these body weights however, were taken over a very wide range of species, from the polar bear (*Ursus maritimus* L.) weighing approximately 100,000 g to the little brown bat (*Myotis lucifugus* Le Conte) weighing only about 8 g. The cottontail, the New Zealand wild rabbit, and the domestic rabbit all fit Christian's graph reasonably well. Judging from the sample available, however, cottontails have heavier adrenals relative to their body weight than have the wild rabbits in New Zealand or domestic rabbits.

Rogers and Richter (1948) were the first to point out the wide divergence in adrenal weights relative to body weights between captive and wild rats (*Rattus norvegicus* and *R. rattus*). As Brown *et al.* (1926) unfortunately did not record sexes separately of his domestic rabbits, the figures given by these authors do not permit of comparison with the data on wild rabbits presented here.

Adrenal Weight of Rabbits in Relation to Population Density and Condition

Christian (1955) and Christian and Davis (1956 (a) and (b)) postulated that population density and endocrine responses are related in mammals. It is also known that population pressure produces adrenal hypertrophy, which is a reaction of the body to stress (Selye 1950): with increasing population density there is an expansion in the adreno-cortical function followed by a decline in reproduction. Christian (1959) recently reviewed all the evidence from laboratory and field experiments with populations of limited size, with freely growing confined populations, and with natural populations of a number of small mammals such as mice (*Mus* sp. and *Peromyscus* sp.), voles (*Microtus* and *Clethrionomys* spp.) and rats (*Rattus* spp.). He concluded that in all species investigated "there is a density-dependent sociopsychological-physiological feed-back system, always operative, which functions as the basic and often only regulator of population growth." Chitty (1955), working with voles in the field and in

the laboratory, brought forward the hypothesis that the numbers of voles are regulated by a non-infectious pathological condition affecting some members in a population.

The data presented here show (Figs 3 and 4) a similar but less pronounced general trend of an increase in adrenal weight with population density in wild rabbits as was shown for other species; this trend is more pronounced in males.

To understand the significance of these findings and to compare them with results of other workers, we shall now consider the correlations between paunched weight and adrenal weight, and between fat content and adrenal weight in various age groups and according to population densities.

It was found that paunched weight did not decrease with the increasing population density; in fact the weight of the young rabbits increased significantly. Similarly, Figs 1 and 2 show a general increase in the mean fat content from sparse to high population and that the fat content of young rabbits in medium and high populations was nearly equal to that of the older age groups. There seems to be a marked similarity between the graphs of fat content (Figs 1 and 2) and those showing the mean adrenal weight (Figs 3 and 4) in the four populations with densities ranging from about 1 rabbit to 5 acres to that of 23 rabbits to an acre. These two variables were tested for correlation; there does seem to be some evidence of difference between the sparse and the high population, although conclusions cannot be drawn with a great deal of confidence. It would appear that within the sparse population the adrenal weight increases as the fat content increases, but within the high population the adrenal weight decreases as the fat content increases.

Summing up, the picture resulting from the present work with wild rabbit populations of different density is unlike that obtained by authors working with other mammal species. Although with increasing population density there is a general increase of adreno-cortical activity as indicated by adrenal weight, this not only does not lead to any noticeable stress but, on the contrary, seems to improve the rabbits' condition as shown by fat deposits. Similarly, Southwick (1958) detected no enlargement of adrenals in either sex in a very dense population of house mice (*Mus musculus*) in English corn ricks; though 45% of the adult males showed signs of severe fighting. Judging by general appearance of the rabbits, no sign of severe fighting was recorded in any of our populations.

It is surprising to find that rabbits at a density of 23 to the acre are in better condition than those at lower densities. Two reasons for this may be put forward: the social habits, and the environmental requirements of the wild rabbit—both having some bearing on practical control problems. The work of Southern (1948) and of Mykytowycz (1958) on rabbit behaviour suggests that social habits may require a certain minimum population density for the well-being of the population. Observations by Howard (1958, pp. 21–24) suggest that in New Zealand in climatic conditions of a medium rainfall, only in dense populations can rabbits produce and maintain the neatly cropped short grass and some bare soil, which seems essential for their well being, unless they are assisted by sheep or fire.

ACKNOWLEDGMENTS

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BARK EXTRACTIVES OF *DACRYDIUM CUPRESSINUM* SOLAND

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Summary

Totarol, β-sitosterol, sequoyitol and a leucoanthocyanidin have been isolated from the bark of *Dacrydium cupressinum*. The leucoanthocyanidin forms a crystalline tetra-O-methyl ether and gives cyanidin when treated with acids. A tetra-O-methyl leucocyanidin derived from (+)-tetra-O-methyl taxifolin by reduction with sodium borohydride differs from the tetramethyl ether of the naturally occurring compound.

Introduction

Previous investigations of the extractives of *Dacrydium cupressinum* Soland. have dealt with the constituents from the wood and the leaves. Easterfield and Aston (1904, 1911) first isolated an acid from the wood resin, which was later shown to be identical with podocarpic acid and McDowall and Finlay (1925) showed that the principal constituent from the essential oil of the leaves is the diterpene, rimuene. A further diterpene from the oil was again isolated by Hosking and shown to be phyllocladene (Briggs, 1937). McDowall and Finlay also obtained a sesquiterpene which was again isolated along with rimuene and α-pinene by Carrie (1932). Brandt and Thomas (1951) investigated the neutral portion of the wood resin and found the constituents to be totarol, ferruginol, sugiol, macrocarpol, a phytosterol which was probably β-sitosterol and a crystalline wax. The acidic portion was again reported to contain a considerable amount of podocarpic acid.

In the present investigation a methanolic extract of the bark was mixed with Celite and re-extracted with solvents of increasing polarity, *viz.*, light petroleum, ether, ethyl acetate, and acetone. The first three extracts were fractionated between alkalies to give acidic, phenolic, and neutral fractions.

The neutral fraction from the light petroleum extract when acetylated and chromatographed on alumina gave a crystalline acetate which was identified as totaryl acetate by comparison with an authentic sample and by deacetylation to totarol. Rechromatography of the oils and mother liquors from the initial column led to the isolation of a further acetate which was identified as β-sitosteryl acetate. β-Sitosterol, obtained by deacetylation was identical with an authentic sample and also with a sample of the phytosterol isolated by Brandt and Thomas from the wood resin, thus confirming the identification of their product.

A further crystalline compound was isolated from the acetone extract after extraction of the concentrate with butan-1-ol followed by water. This was identified as the cyclitol, sequoyitol, by comparison with an authentic sample and preparation of the penta-acetate. No crystalline products were isolated from the ether and ethyl acetate extracts or in a search for resin acids in acidic fractions.

The acetone extract contained considerable amounts of red amorphous tannin-like material which was readily polymerised by the action of dilute acids, and from which glucose was identified, chromatographically, in the aqueous hydrolysates. The polymer was non-mobile on paper chromatograms and was markedly astringent. Aston has examined the bark for tannin content (see Briggs, 1947) and records a yield of 10%. Bate-Smith and Swain (1953, 1956), however, have pointed out that many plant tissues which hitherto have been supposed to contain condensed tannins on the basis of colour tests and phlobaphene reaction actually contain leucoanthocyanins. Their occurrence, especially of those yielding cyanidin and delphinidin on acid treatment, is widespread but confined mainly to "woody" plants. In the present investigation the acetone extract from a fresh sample of bark was therefore examined for leucoanthocyanidin content. By working up the extract along similar lines to those used by Ganguly and Seshadri (1958), Ganguly *et al.* (1959) an amorphous leucoanthocyanidin fraction was obtained which was homogeneous when examined by paper chromatography but which could not be obtained crystalline. It could also be isolated in amorphous form by preparative chromatography of crude fractions on filter paper. When converted to the pyrylium salt it gave cyanidin chloride, identified by co-chromatography in three solvent systems. No sugars were detected in the hydrolysates and the compound was therefore one of the possible stereoisomeric forms of leucocyanidin (5:7:3′:4′-tetrahydroxyflavan-3:4-diol). The acetate could not be obtained crystalline but when the leucocyanidin was treated with diazomethane in alcoholic solution it formed a crystalline laevorotatory tetra-O-methyl ether, melting point 172°–174°.

Leucocyanidins have previously been isolated from the gum of *Butea frondosa* (Ganguly and Seshadri, 1959), tamarind seed testa (Laumas and Seshadri, 1958) and *Bruguiera gymnorrhiza* (Seshadri and Venkataramani, 1959). The tetramethyl ether of that from *Butea frondosa* is apparently an optically active form of one of two racemic isomers obtained by the reduction of tetra-O-methyl taxifolin with sodium borohydride. In the present work repetition of Seshadri's reduction of tetra-O-methyl taxifolin [obtained from (+)-taxifolin of known optical purity from *Podocarpus spicatus* (Briggs, Cambie and Hoare, 1959)] resulted in the formation in major yield of a single tetra-O-methyl leucocyanidin. Moreover the melting point of this derivative (206°–208°) is higher than that of either of the two isomers obtained by Seshadri (172° and 198°, respectively) and differs considerably from that (123°–125°) of a similar derivative prepared by Swain (1954) by the same method. The melting point, however, does agree more closely with the melting point (204°–206°) for the methylated product obtained by Freudenberg and Weinges (1958) from a racemic leucocyanidin derived from taxifolin. Lack of (+)-taxifolin precluded a repetition of the experiment but reduction of (+)-tetra-O-methyl taxifolin [obtained from (+)-taxifolin by acid hydroly-

sis of astilbin from *Quintinnia serrata* (Cambie, 1959) and simultaneous racemisation] again gave, as the major product, a single compound, melting point 178°–179°. The infra-red spectra of these tetra-O-methyl leucocyanidins were identical but differed from that of the methyl ether of the naturally occurring compound from *Dacrydium cupressinum*. As the rotation of this latter derivative is of opposite sign to any of the previously recorded ethers of natural leucocyanidins it would appear that our compound is another of the eight theoretically possible stereoisomers of leucocyanidin.

EXPERIMENTAL

Analyses are by Dr A. D. Campbell, University of Otago, New Zealand. Infra-red spectra were measured in KBr discs and ultraviolet spectra were measured for EtOH solutions. Alumina was of activity II (Brockmann). Light petroleum was of b.p. 60°–80°.

EXTRACTION OF *Dacrydium cupressinum* BARK

The bark (900 g) collected from mature trees in the Waitakere Ranges was continuously extracted (Soxhlet) in portions with methanol, the extract concentrated, *in vacuo,* and the dark syrup so obtained, intimately mixed with Celite. The finely powdered and dried solid was then successively re-extracted with light petroleum, ether, ethyl acetate and acetone, each for 24 hr. The concentrates from the light petroleum, ether and ethyl acetate extracts were fractionated between saturated aqueous sodium hydrogen carbonate, 10% sodium carbonate and 10% sodium hydroxide.

TOTAROL

Solvent was removed from the neutral fraction obtained from the light petroleum extract and the total oil (3·1 g) acetylated with acetic anhydride (20 cc) and pyridine (10 cc) for 3 hr at 100°. The viscous gum obtained on pouring the reaction mixture into ice-water was dried, *in vacuo,* and chromatographed in benzene on alumina. Fractions eluted from the column with benzene (total vol 600 cc) on concentration and long standing deposited crystals mixed with yellow oil. Repeated recrystallisation from ethanol gave totaryl acetate (1·4 g) as large prisms, m.p. and mixed m.p. 125°–126°, $[\alpha]^{20}_{D}$ + 44° (*c* 1·1 in EtOH). [Found: C, 80·3; H, 9·7; Ac, 13·8. Calc. for $C_{22}H_{32}O_2$: C, 80·4; H, 9·8; 1 Ac, 13·2%] $\lambda_{max.}$ 265 mμ (log ξ 2·8). The infrared spectrum was identical with that of an authentic sample.

Deacetylation was effected by heating the acetate (100 mg) under reflux with 2N-methanolic potassium hydroxide for 1 hr. The product, isolated from the reaction mixture by ether extraction, crystallised from light petroleum to give prisms of totarol (75 mg), m.p. and mixed m.p. 132°, $[\alpha]^{20}_{D}$ + 42° (*c* 1·5 in EtOH). [Found: C, 83·6; H, 10·7. Calc. for $C_{20}H_{30}O$: C, 83·9; H, 10·6%]. The infrared spectrum was identical with that of an authentic sample.

β-SITOSTEROL

The mother liquors from totaryl acetate and further yellow oils eluted from the column above with ether and with benzene-ether mixtures were

combined and rechromatographed in light petroleum on alumina. Fractions eluted with the same solvent and with benzene gave a solid which crystallised from methanol to yield β-sitosteryl acetate (120 mg) as glistening plates, m.p. and mixed m.p. 126°–128°, $[\alpha]^{20}_D$ — 38·1° (*c* 0·9 in $CHCl_3$). [Found: C, 81·8; H, 11·9. Calc. for $C_{31}H_{52}O_2$: C, 81·5; H, 11·4%].

Deacetylation in the above manner and isolation of the product by the addition of water gave β-sitosterol which crystallised from ethanol as needles, m.p. and mixed m.p. 138°–139°. The infrared spectrum was identical with that of an authentic sample.

SEQUOYITOL

The residue from a portion of the acetone extract (from 800 g bark) was repeatedly extracted with hot butan-1-ol and the combined extracts concentrated, *in vacuo*. The resulting dark tar was re-extracted with hot water (3 × 100 cc) and the extracts concentrated, *in vacuo*, to yield a yellow syrup which on long standing in the presence of ethanol gave needles of sequoyitol (116 mg), m.p. and mixed m.p. 238°–239°. [Found: C, 43·8; H, 7·4. Calc. for $C_7H_{14}O_6$: C, 43·3; H, 7·3%]. The infra-red spectrum was identical with that of an authentic sample.

Acetylation of sequoyitol (50 mg) with acetic anhydride-pyridine (100°, 1 hr) gave the penta-acetate (48 mg), which formed needles, m.p. and mixed m.p. 198°–200°, from benzene-ligroin.

LEUCOCYANIDIN

The residue from a further acetone extract (from 1·5 kg of fresh bark), stripped of chloroform solubles, was extracted with hot (60°) water (3 × 30 cc) and the aqueous solution extracted with methyl ethyl ketone (2 × 25 cc), the extracts dried (anhydrous Na_2SO_4) and the solvent removed, *in vacuo*, to yield a crude leucocyanidin fraction (30 g), circular R_f 0·59 (6% aqueous acetic acid), circular R_f 0·61 (saturated aqueous butan-2-ol). Spots were detected by the use of ferric chloride-ferricyanide spray reagent or by 5% ethanolic p-toluene sulphonic acid (Roux, 1957) followed by heating at 100° for 5 min. Further purification was effected by the method of Ganguly and Seshadri or by preparative chromatography on Whatman No. 3 paper with 6% acetic acid to give the leucoanthocyanidin as a pale pink amorphous solid which darkened at 180°, turned black at 250° but which showed no true m.p. below 360°. The compound rapidly polymerised in the presence of light and satisfactory analyses were not obtained. The product was insoluble in light petroleum, ether and chloroform but readily soluble in water, methanol and ethyl acetate. With alcoholic HCl it developed a red colour in the cold which deepened on heating and with alcoholic ferric chloride gave a green colour which darkened on standing.

CONVERSION TO CYANIDIN CHLORIDE

Heating the leucocyanidin (15 mg) at 100° for 1 hour with 2N-hydrochloric acid and propan-1-ol in sealed tubes converted the compound to the pyrylium salt. The deep red solution was evaporated, *in vacuo*, extracted with boiling 15% aqueous 0·1N-methanolic hydrochloric acid, the filtered solution saturated with sodium chloride and re-extracted with a few drops of

pentan-1-ol. The pigment was identical with cyanidin chloride, prepared from quercetin by the method of King and White (1957), when examined by co-chromatography, circular R_{fs} 0·67 (Forestal solvent), 0·76 (BuOH-2N HCl), 0·53 (m-cresol-2N HCl). Spots of pigment and cyanidin chloride had the same appearance after spraying with 0·1M – aluminium chloride spray reagent.

TETRA-O-METHYL LEUCOCYANIDIN

Treatment of the leucocyanidin in methanol solution with diazomethane in the cold gave the *tetra-O-methyl ether* which crystallised from methanol as prisms, m.p. 172°–174° $[\alpha]^{20}_D$ — 45·6° (*c* 0·2 in EtOH). [Found: C, 62·8; H, 6·05; OMe, 34·7. $C_{19}H_{22}O_7$ requires: C, 63·0; H, 6·1; 4OMe, 34·2%]. It was insoluble in aqueous alkali and gave no colour with ferric chloride.

REDUCTION OF (+)-TAXIFOLIN TETRAMETHYL ETHER

Methylation of taxifolin (90 mg, $[\alpha]^{25}_D$ + 48° (*c* 1·0 in 50% aqueous Me_2CO), dried over magnesium perchlorate, by the method of Hergert, Coad, and Logan (1956) gave the tetra-O-methyl ether (72 mg), needles, m.p. 164°–166°, from ethanol, $[\alpha]^{20}_D$ — 23·8° (*c* 0·8 in $CHCl_3$). Clark-Lewis and Korytnyk (1958) record m.p. 165°–168° and $[\alpha]^{16}_D$ — 23·4°. An ice-cold solution of the methyl ether (70 mg) in methanol (5 cc) was treated with sodium borohydride (30 mg) and the solution stood at room temp. for 24 hr. A crystalline precipitate which separated from the reaction mixture crystallised from methanol to give a tetra-O-methyl leucocyanidin (62 mg) as rectangular rods, m.p. 206°–208°. [Found: C, 62·6; H, 5·4. Calc. for $C_{19}H_{22}O_7$: C, 63·0; H, 6·1%]. No further crystalline product was obtained from the mother liquors.

Similar reduction of (±)-taxifolin tetra-O-methyl ether (3 g), m.p. 168°–169° prepared from (±)-taxifolin in the above manner (Clark-Lewis and Korytnyk record, m.p. 169°–170°), afforded a tetra-O-methyl leucocyanidin (2·8 g), m.p. 177°–178°. [Found: C, 63·2; H, 6·0%]. No further crystalline products were obtained.

ACKNOWLEDGMENTS

The authors thank Dr S. J. Angyal, University of New South Wales, for a sample of sequoyitol and Dr R. C. Cooper, Auckland Institute and Museum for identification and assistance in collection of the bark. One of the authors (R.C.C.) gratefully acknowledges assistance from the Research Grants Committee of the University of New Zealand.

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AN APPLICATION OF NUCLEAR MAGNETIC RESONANCE TECHNIQUES TO STUDY THE SORBED WATER IN CLAYS

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Summary

Nuclear magnetic resonance is shown to be a useful technique for investigating the nature of the sorbed water in clays. Preliminary experimental results are discussed, the main conclusions of which are (1) for kaolin the amount of free surface water is small and the bound surface water is fairly loosely bound to the clay surface, (2) for montmorillonite most of the interlayer water is loosely bound with little intermolecular interaction.

Introduction*

It is well known that some clays have the property of holding water in various ways, in the form of layers between sheets of the parent substance, adsorbed on the external surfaces, or bound within the structure in the form of OH groups. This is illustrated in Fig. 1 for kaolin and montmorillonite at room temperature.

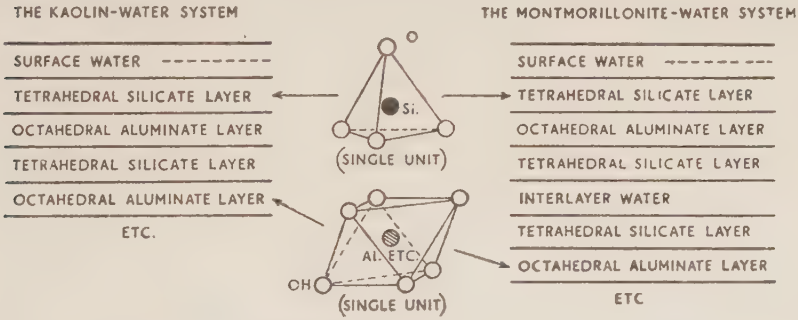

Fig. 1—A diagrammatic representation of the clay-water system for kaolin and montmorillonite.

The surface water has been shown to be in a physical state different from that of ordinary water, as it is denser and more viscous. This would apply only to the water which is actually close to the surface, say of the order of 3 to 10 molecular layers. Any additional water would tend to have its usual properties. The most likely structure of the surface water is that in which the initially adsorbed water has an ice-like crystalline form. This comes about because of the similarity between the positions of the oxygen atoms in ice

*Grim (1953) discusses alternative theories of the structure of the sorbed water and gives references to the experimental investigations.

and those exposed at the clay surface. Although there has been a certain amount of evidence from electron diffraction studies and density measurements which tends to support the ice-like structure, the actual form of the surface water layers is still undetermined.

It is expected that at the interlayer surfaces the water molecules would be bound and have a similar structure to that of the surface water. In the interior of the interlayers the water molecules may have a crystalline form, or they could be free to move about as in ordinary water. Unlike the surface water, which requires little energy for its complete removal, the interlayer water requires a definite amount of energy before it can be expelled. X-ray diffraction measurements show that the variable thickness of the interlayer water is always an integral multiple of the diameter of the water molecule.

Many attempts have been made to determine the nature of the interlayer and surface water. These have included the methods of infra-red spectroscopy, electron diffraction, differential thermal analysis and dehydration studies. The last two mentioned have involved the study of changes caused by liberation of the water as the temperature is increased. The general behaviour is for the surface water, which is the least bound, to be evaporated at a temperature just above that of the room and more rapidly at higher temperatures. At about 100°C the interlayer water is removed if sufficient time is allowed, but it is given off more readily at higher temperatures. If the temperature is further increased the lattice water is liberated and the structure collapses. However, these methods have not been able to yield much information on the actual structure of the sorbed water. It was therefore decided to make a survey of the possibility of using nuclear magnetic resonance techniques to investigate the clay-water system.

The Phenomenon of Nuclear Magnetic Resonance

If a system of isolated magnetic nuclei is in a steady magnetic field the nuclear spin axes will be aligned with respect to the field in certain definite directions depending on their spin quantum number. Corresponding to each alignment direction the nuclear magnet will have certain values of potential energy. Thus the effect of the magnetic field on the nuclei is to establish a system of energy levels, the number and relative spacing of which depend on the spin quantum number and the field strength. When the nuclei are in thermal equilibrium with one another the relative population of the energy levels is determined by a Boltzmann distribution. If now a quantum of energy in the form of $h\nu$, which is just the energy difference between the adjacent levels, is applied to the system transitions can occur from a lower level to the next highest. For protons in a magnetic field of strength 2,500 gauss the resonance frequency ν is approximately 10·7 Mc/s. Unless the nuclear spin system has some means of losing energy, or cooling off, so that the original population excess of the lower energy levels can be restored, there would very soon be no absorption of radio frequency energy.

For a diamagnetic material the dominant mode of coupling between the nuclear spin system and its surroundings (the lattice) is provided by the interaction between the nuclear magnets and the fluctuating magnetic fields

of the lattice. These fields are caused by local magnetic nuclei which are transported about by the thermal movements of their atoms and molecules. Thus interaction or "relaxation" between nuclei and lattice can occur when the spectral distribution function describing the thermal motions has a finite value at the resonance frequency. The effectiveness of the spin-lattice interactions can be described by a single parameter T_1, the spin-lattice relaxation time, which is the time constant at which the hot spin system returns to thermal equilibrium with the lattice. For many kinds of molecular motion T_1 can be found in terms of a single correlation time which can be regarded as that time for which a molecular configuration can persist about a given nucleus.

The identical nuclear spins remain in thermal equilibrium with one another by the exchange of quanta at the resonance frequency. In a similar manner to that of the spin-lattice relaxation this process is described by a spin-spin relaxation time T_2. The static magnetic field at a nucleus is modified from the value of the external field by the local field of adjacent magnetic nuclei. This local field is characteristic of the type and configuration of the surrounding nuclei and depends on the kind of molecular motion of the substance. Both of these spin-spin interaction effects modify the resonance spectrum by altering its shape and width.

The amount of radio frequency energy applied to the sample is usually kept below a certain level (depending on the $T_1 \cdot T_2$ product) to avoid saturation effects which reduce the resonance intensity.

Liquids are characterised by the rapid Brownian motion and rotational motion, or tumbling, of their molecules. By finding the correlation time for the random molecular motion in water Bloembergen *et al.* (1948) calculated its T_1 and T_2 as a function of viscosity and temperature. At lower temperatures the width of the resonance spectrum depends mainly on the spin-spin interaction between the two protons in the water molecule (O^{16} has no magnetic moment). Figure 2 shows the theoretical variation of the relaxation times with correlation time.

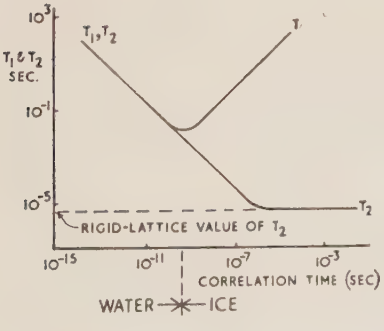

FIG. 2—The relaxation times as a function of correlation time (after Bloembergen *et al.*, 1948).

The nuclear magnetic resonance absorption signal for solid samples is usually much broader and weaker than is the case for liquids because of the restricted molecular motion. When the amplitude and frequency of the molecular motion are so small that it has a negligible effect on the spectrum the lattice is said to be "frozen". In this condition the structure of the resonance line is completely determined by the local field of the adjacent nuclei. The kind of molecular motion can be determined from variations of line shape with temperature. Andrew (1955) and Wertz (1955) cover these topics more fully.

STUDIES OF SORBED WATER USING NUCLEAR MAGNETIC RESONANCE

The total water content of a substance is made up from two main sources.

1. *The Absorbed Water in the Body of the Hygroscopic Substance:* In this case the water molecules are not rigidly bound into the structure as water of hydration but neither are they as free as those in ordinary liquid water. The resonance line width is therefore much broader than that of liquid water but is usually far narrower than that of a hydrogen containing solid absorbent. Shaw and Elsken (1953) found, by using substances of a biological origin, that above a certain concentration and at a constant temperature, the resonance amplitude is proportional to the amount of absorbed water. Below this critical amount, which depends on the nature of the absorbent, the initially absorbed water is tightly bound to the inner surfaces of the material. This has the effect of restricting the motion of the water molecules causing the resonance intensity to depart from the linear concentration relationship. At low concentrations the line broadens rapidly and approaches a "rigid lattice" value.

2. *The Adsorbed Water:* Mays and Brady (1956) show that for very low coverage, i.e. less than a monolayer, the water molecules are bound to the surface and do not have much interaction with one another. The line widths will be determined by intramolecular proton-proton interaction in the H_2O molecule. In this case the line width at room temperature will be greater than that of liquid water. As the temperature is reduced the line width broadens only slightly. These results indicate that the water is fairly loosely bound to the surface so that motion can persist strongly down to low temperatures.

If the coverage is increased to the order of a complete monolayer, interaction between adsorbed molecules increases and the structure becomes crystalline in form. This restricts motion so that the line width at low temperatures will be about the same as that of ice. As the number of surface layers is increased the behaviour of the outer layers approaches that of ordinary water.

EXPERIMENTAL APPARATUS AND METHODS

The basic experiment is to observe the absorption (or emission) of energy at the resonance frequency when the nuclear sample is in a magnetic field. The observables in nuclear magnetic resonance are the same as those in any other form of spectroscopy, namely (1) the position of the resonance peaks in the frequency spectrum (in the simplest case the resonance frequency), (2) the line width and shape, and (3) the line intensity.

The field was provided by a modified magnetron permanent magnet with an outside pole diameter of 4 in. and a 39° pole cap taper. The central field strength was 2,500 gauss when the gap had its usual value of 1 inch. By observing the strong resonance signal from a paraffin oil sample the field within the gap was explored and the pole gap geometry adjusted until a practical limit to the field uniformity was attained. Using ring shims of

Andrew and Rushworth (1952) type the field variation in the most uniform part of the field was 0·60 gauss over a 1 cm^3 volume. The low field strength of the magnet and only moderate uniformity, by nuclear resonance standards, is the ultimate limitation of the apparatus.

From the varieties of detecting systems that have been described the marginal oscillator method of Pound and Knight (1950), Pound (1952) was adopted as being the most suitable for the type of investigations undertaken. A low magnetic field modulation frequency of 20 c/s was used to avoid the interference of induced emf in the sample coil from the modulation field. Pound and Knight (1950) chose a high-frequency of 420 c/s to overcome flicker effect noise in the oscillator which becomes more serious, compared with other noise sources, at low modulation frequencies. They require an additional field to cancel out the induced emf. However, using a wave analyser with a 4 c/s bandwith no variation in noise output could be found over a frequency range of 20 c/s to 2 kc/s. More recently Robinson and Geiger (1958) with a similar apparatus have used a modulation frequency of 30 c/s and they make no mention of the flicker effect.

Restricted working space inside the magnet gap necessitated a simple form of temperature control. As a Dewar vessel was unsuitable for holding a cooling mixture because of its necessary bulk, use was made of a single walled glass container shaped to fit between the poles. To avoid reducing the Q of the sample coil by surrounding it with a copper shield, which is necessary when hydrogen containing coolants are used, a carbon disulphide dry ice mixture or carbon disulphide cooled with liquid oxygen, was allowed to come into direct contact with the R.F. coil. Carbon disulphide is an almost ideal material for this purpose because it contains no hydrogen, so that proton resonance can be observed in the sample without interference, it does not load the tuned circuit, it is a fairly good thermal conductor and has a low freezing point of $-110°C$. Its disadvantages are high inflammability and unpleasant smell, both of which are of secondary importance. Lower temperatures were achieved by the use of liquid oxygen alone. A copper-constantan thermocouple was embedded in the sample to measure its temperature. Although the method of temperature control was not ideal it was adequate for the type of measurements undertaken.

The sample could be heated by two heating coils; one, which was maintained at a slightly higher temperature, was located below the R.F. coil and the other above it so that there was a small but sufficient temperature gradient over the sample tending to force the water out and to prevent it from running back into the sample after condensation on cooler parts of the specimen holder.

Even with the limitations of the present apparatus, due fundamentally to deficiencies of the magnet, useful results can be obtained. As an example the proton resonance in ammonium biflouride (NH_4HF_2) was observed down to a temperature of 110°K. A line width change at approximately 120°K was interpreted as evidence for the hindered rotation of the NH_4^+ tetrahedra. The potential barrier against rotation was found to be 4·0 k cal/mole.

A more complete description of the apparatus and techniques used is given by the author in his thesis (Sears, 1958).

RESULTS FOR CLAY SAMPLES

(a) *Kaolin:* The samples, which had a well formed structure, came from McNamee mine, Langley, U.S.A.

At room temperature, with an undried sample, there was evidence of a two component line structure. When the R.F. voltage across the sample coil was increased the narrow line component was found to saturate before the broad component.

Measurements were also made on a sample of gibbsite $Al(OH)_3$ which has a layer structure composed of double sheets of closely packed hydroxyl groups in which aluminium atoms occupy two-thirds of the octahedral vacancies between the two layers (Wells, 1950). The resonance line of gibbsite is approximately twice as wide as that of the broad component of the kaolin spectrum. It is much weaker and is more easily saturated which is common if the structure is rigid, i.e. the $T_1 \cdot T_2$ product is large. The gibbsite structure is known to be rigid at room temperature from an experiment in which a sample was cooled.

Thus it was concluded that the structural OH groups in kaolin were not making a significant contribution to the resonance spectrum. The broad line is attributed to the bound surface water whose $T_1 \cdot T_2$ product is less than that of the free surface water which is responsible for the narrow line component.

After drying the kaolin sample over concentrated sulphuric acid for four days no change in the resonance spectrum could be seen, but after six days the narrow line disappeared showing that the free water had left the clay surface.

A temperature run was taken on the sample which had been dried for four days, and the results are given in Fig. 3 (a). The experimental errors are the maximum possible uncertainties in the measurements. The narrow line width remained fairly constant with temperature until the free water component became too weak to observe, i.e. the spin-lattice relaxation time had increased because of reduced molecular motion. There is no sudden change in the region of the freezing point of water. This is direct evidence that the amount of free surface water on kaolin is small because there cannot be much interaction between the molecules. These observations support the conclusions of De Wit and Arens on the quantity of adsorbed water on kaolin rather than those of Jaeger and Spiel (Grim, 1953, pp. 174, 175).

As expected the bound surface water line component does not show much variation with temperature. This is confirmed by the results for the sample which had been dried for six days. In this case the sample temperature was reduced to 110°K (Fig. 3 (b)). According to the interpretation of Mays and Brady (1956) the results indicate that the water is fairly loosely bound to the clay surface.

(b) *Montmorillonite:* The samples were British Pharmacopoeia grade bentonite which is largely composed of montmorillonite.

No definite evidence of a two component line structure could be observed. A temperature run on an undried sample was taken between room temperature and 103°K. The results, which are plotted in Fig. 4 (a), show a definite line width change.

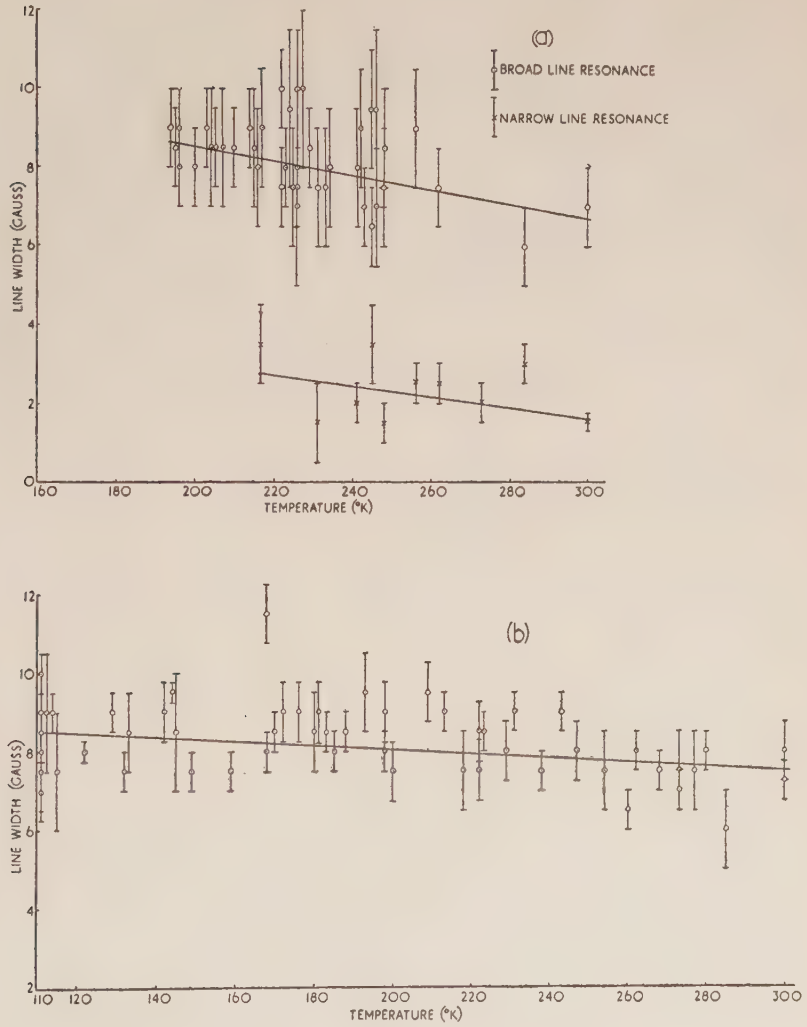

FIG. 3—Line width variation with temperature for kaolin (a) after four days' drying, (b) after six days' drying.

Most of the water in montmorillonite is held between layers but it is capable of retaining more surface water than kaolin. Thus the room temperature line shape should be the resultant of the spectra for the surface (free and bound) and interlayer water components. This resultant is closer to the free water line width of kaolin than to the bound water value which means that the free or more loosely bound water must predominate. At low temperatures the free water component of the line spectrum will be less effective because of its reduced molecular motion. Hence in this region the resonance

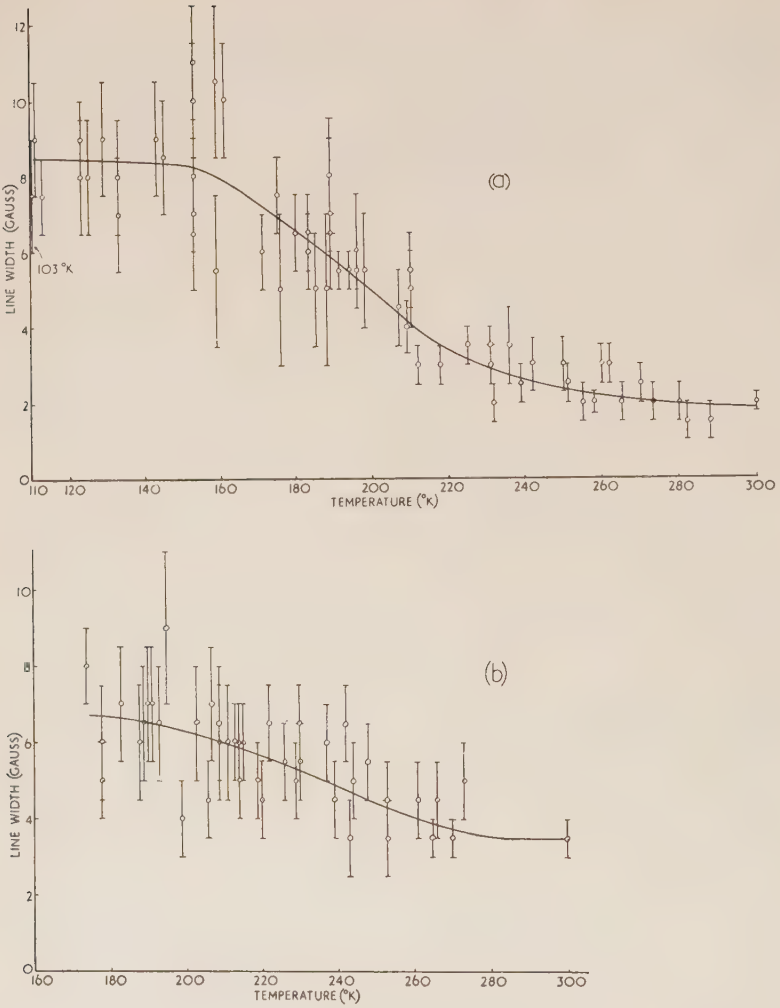

FIG. 4—Line width variation with temperature for montmorillonite (a) undried, (b) dried for three days.

spectrum should be caused mainly by contributions from the bound components.

The line width variations for a sample which had been dried for three days are shown in Fig. 4 (b). The temperature was only reduced to 160°K as the previous results showed that there were no further variations below this temperature. The variation of line width with temperature is much less than that of the undried sample. It is concluded that most of the interlayer water in montmorillonite is loosely bound with little interaction between its molecules.

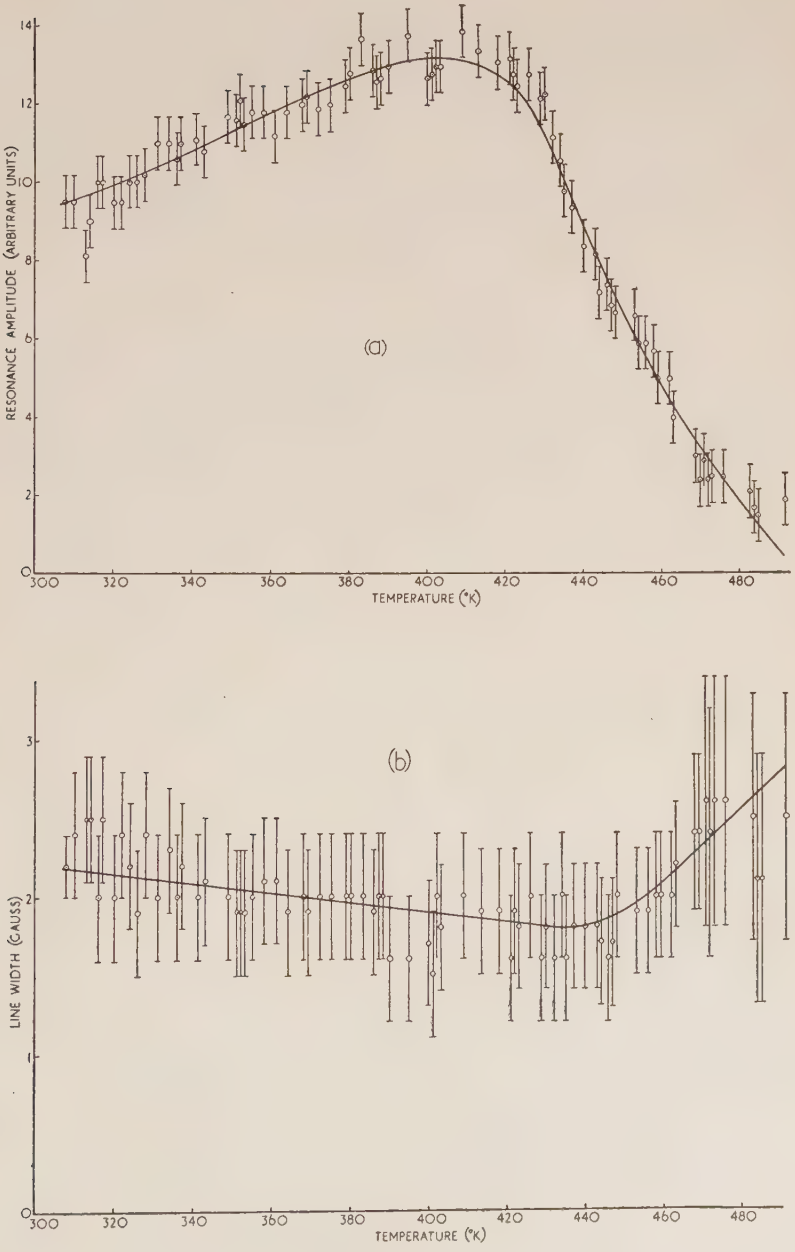

FIG. 5—Heating up results for undried montmorillonite (a) resonance amplitude versus temperature, (b) line width versus temperature.

After a further drying for three days there was no change in the resonance line structure at room temperature.

An undried sample was heated over a period of six hours to 500°K and the resonance line width and amplitude measured. Fig. 5 (a) shows how the amplitude increased to a maximum at approximately 400°K and decreased rapidly at higher temperatures. In the vicinity of 435°K there was audible indication that the interlayer water was being released. Above 500°K, when all of the interlayer water had left the sample, the resonance of the lattice OH water could not be detected. The line width-temperature curve Fig. 5 (b) has a broad minimum at about 430°K and a steep rise thereafter.

At first it was expected that the line width changes would show three distinct transitions with the successive liberation of the free surface water, the bound surface water and the interlayer water. The results, however, show only the change which occurred when the interlayer water was given off, the effects of the removal of the surface water being more than compensated for by the increased molecular motion of the remaining water. This indicates that, for the sample used, the interlayer water is far more predominant than the surface water. Because the line width results reveal no major change before 430°K there could not have been a breakdown of the structure of the interlayer water, as from an ice like structure to a more liquid form, before its liberation from the clay. The conclusion is, as before, that most of the interlayer water is in a relatively free state.

ACKNOWLEDGMENTS

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SEASONAL CHANGES IN DIATOMS AND DINOFLAGELLATES OFF THE EAST COAST OF NEW ZEALAND DURING 1957 AND 1958

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Summary

In a survey of seasonal fluctuations of diatom and dinoflagellates, 147 jar and net samples were collected between July 1957 and July 1958 from 10 stations in the Hauraki Gulf, Wellington Harbour, and Cook Strait. Jar samples taken from the sea surface were centrifuged, and counts made of every plant and animal species that could be identified in the concentrate. Net samples down to 20 metres gave a picture of the major constituents of the subsurface phytoplankton. The Hauraki Gulf emerged as the most prolific diatom locality with at least a moderate population during most times of the year. Both spring and autumn diatom maxima were demonstrated. Dinoflagellates, though less abundant, rose to prominence in late spring and early summer below the surface. In Wellington Harbour diatoms also predominated, but autumn production greatly exceeded that in spring, and surface species disappeared abruptly in May. Southeastern Cook Strait provided a wealth of animal species – particularly in summer – with diatoms scarce to moderate in autumn and winter, and a distinctive dinoflagellate population in midsummer.

Introduction

Apart from the work of Crawford (1947) in Cook Strait and Brewin (1952) in Otago Harbour, the nature and seasonal abundance of phytoplankton in New Zealand coastal waters has been virtually unknown. The present survey, involving a quantitative and qualitative examination of samples mainly from the Hauraki Gulf, Wellington Harbour, and the southeastern part of Cook Strait, was undertaken to obtain a concurrent picture of seasonal changes in different latitudes and in contrasting ecological environments. Between July 1957 and July 1958, 147 samples were collected from the following ten stations (see Fig. 1 (a) and (b) and list of stations, p. 140).

Five of these stations provided sufficient information to trace the variation in dominance of diatoms and dinoflagellates from month to month. Samples in the Hauraki Gulf were collected by members of the crew of the Marine Department fisheries patrol vessels *Clematis* and *Ocean Star*. In Wellington Harbour the Police Launch was made available for fortnightly to monthly collections. The material from Stations 7–10 was supplied through courtesy of Mr F. Abernethy from the fishing trawler *Thomas Currell*. Each sample consisted of one 2 lb jar of surface seawater (800 ml.) and one N50 net vertical haul, from a depth of 20 metres to the surface. Since the samples were usually collected some time before examination, preservation in 3–5% neutral formalin was essential, even though such a treatment destroys all the unarmoured dinoflagellates and the smallest microflagellates of the nannoplankton.

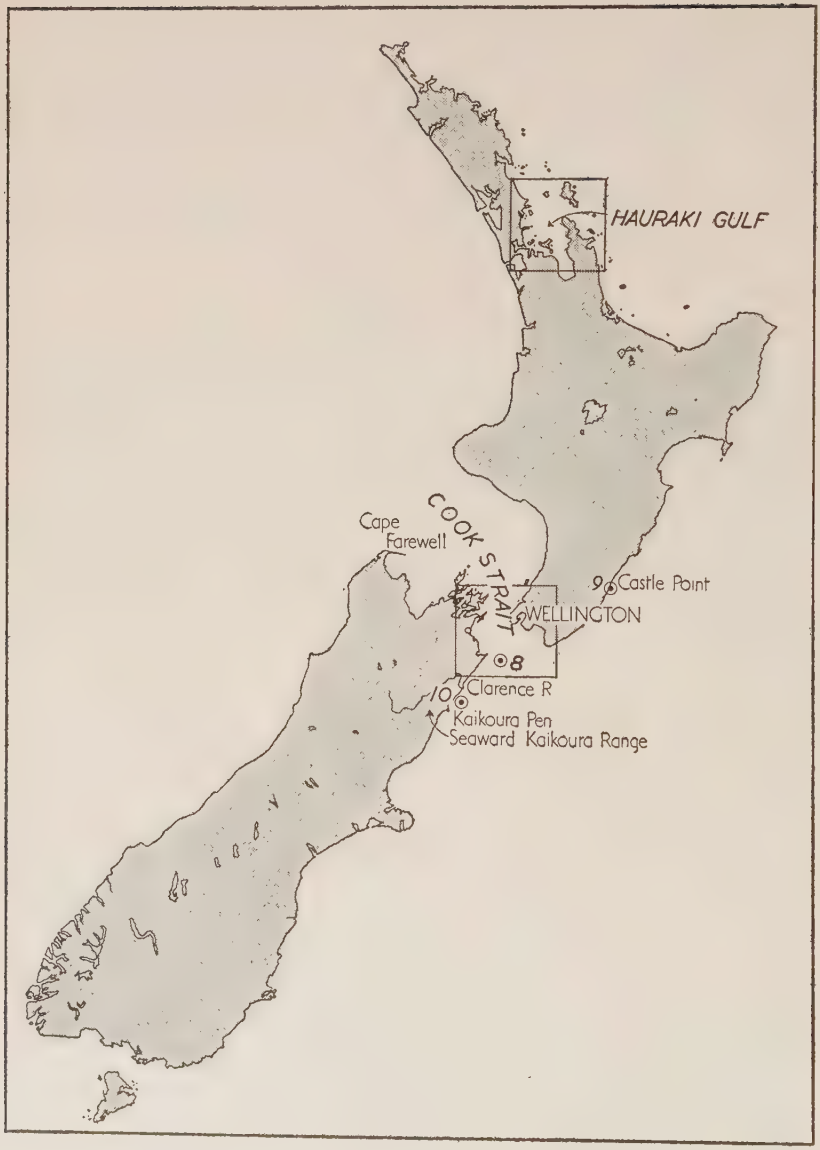
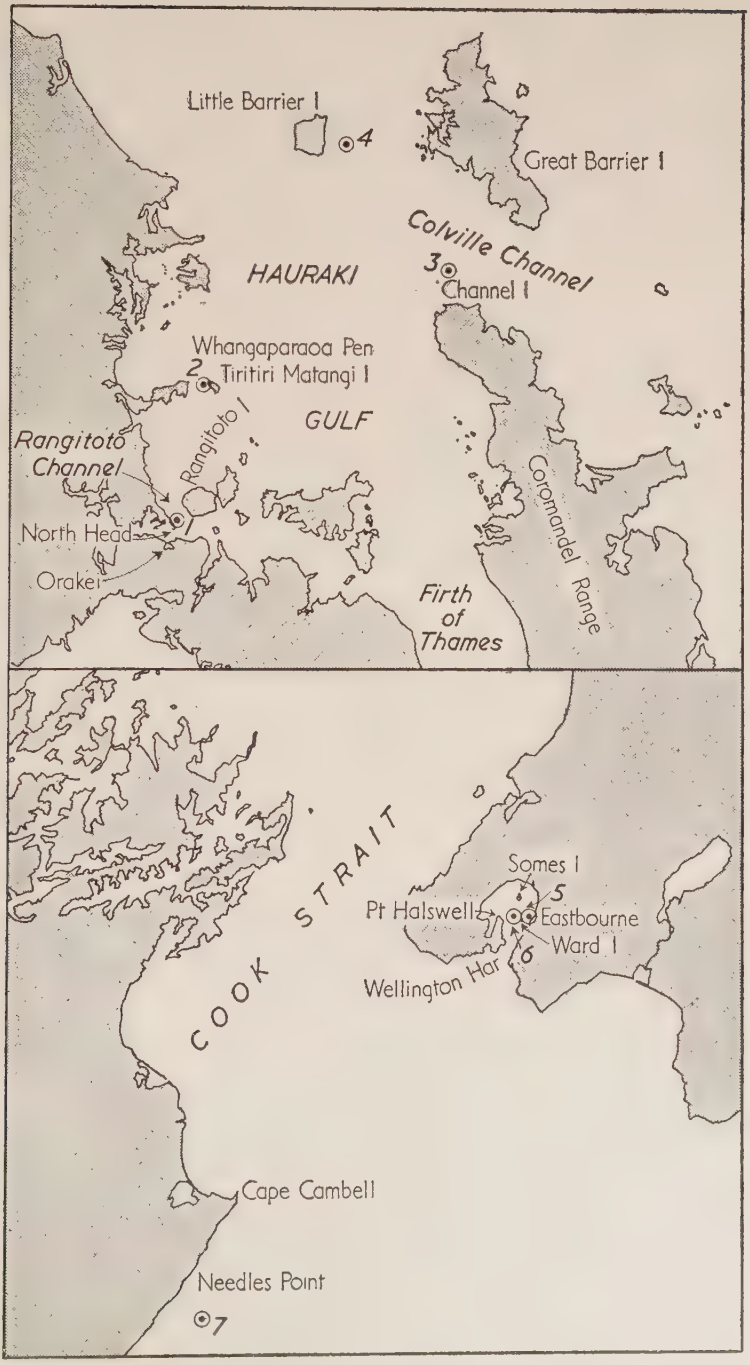


FIG. 1 (a).

FIG. 1—Locality map indicating stations mentioned in the text (a) 8–10, (b) 1–7.


Little Barrier I
4
Great Barrier I
Colville Channel
HAURAKI
3
Channel I
Whangaparaoa Pen
2
Tiritiri Matangi I
GULF
Rangitoto I
Rangitoto Channel
North Head
1
Orakei
Firth of Thames
Coromandel Range
COOK STRAIT
Somes I
5
Pt Halswell
Eastbourne
Ward I
6
Wellington Har
Cape Cambell
Needles Point
7

LIST OF STATIONS

Station	Hauraki Gulf	Location	No. of Samples
*1.	E. of North Head, S. end of Rangitoto Channel	36° 49′S 174° 49′E	23
2.	Midway between Tiri Tiri I. and Whangaparaoa Heads	36° 36′S 174° 52′E	19
3.	Colville Passage, ½-mile E. of Watchman I.	36° 25′S 175° 21′E	10
4.	Little Barrier I., 2 miles E.N.E. of S.E. corner	36° 13′S 175° 9′E	4
	Wellington Harbour (Port Nicholson)		
5.	Midway between Somes I. and Eastbourne Pier	41° 17′S 174° 53′E	23
6.	Midway between Pt. Halswell and Ward I.	41° 17′S 174°51′E	23
	S.E. Cook Strait		
7.	15 miles S.S.W. of Cape Campbell. 3 miles S.E. of Needles Pt.	41° 55′S 174° 12′E	20
8.	13 miles E.S.E. of Cape Campbell.	41° 47′S 174° 31′E	10
	S.E. of North Island		
9.	Castle Point	40° 54′S 176° 14′E	7
	N.E. of South Island		
10.	Midway between Kaikoura Peninsula and Clarence River	42° 18′S 173° 53′E	8

*Stations 1–4 are N.Z.O.I. Stations Z532–Z535.
Stations 5–6 are N.Z.O.I. Stations B198–B199.
Stations 7–10 are N.Z.O.I. Stations Z536–Z539.

At Stations 5–10, temperature and salinity records were kept. Salinity determinations were made by conductivity (Hamon 1956) and gravimetric (Gilmour 1958) methods. Temperatures were obtained to an accuracy of 0·5°F from surface seawater drawn up in a bucket.

CURRENTS AND HYDROLOGY

Accounts by Garner (1954, 1959 a, b), Bary (1959) and Brodie (in press) supply detailed information on the current pattern in New Zealand waters. The salient features of eastern North Island waters and northeast South Island waters can be summarised as follows. Off the North Island east coast warm and highly saline subtropical water of the East Auckland and East Cape Currents flows southward. The Hauraki Gulf is directly influenced by the East Auckland Current. A tongue of the East Cape Current frequently penetrates south of Castle Point, usually some distance offshore. This tongue may swing westward into the eastern part of Cook Strait.

Subantarctic water moves northward along the Kaikoura coast as far as Castle Point, occasionally reaching Hawke Bay (the Canterbury Current). In addition there are two centres of cold upwelling water between Cape Campbell and Kaikoura. The inshore position of the subtropical convergence off the North Island east coast fluctuates between Cape Kidnappers and Palliser Bay. Mean annual temperature between Hawke Bay and Kaikoura is given by Garner (1959a) as 12·2°C, nearly 6°C lower than that between North Cape and the Hauraki Gulf (17·8°C). In Table 1 is listed the known range of variation in temperature and salinity of water affecting the stations selected for the present study.

Station 1, in relatively enclosed inner waters of the Hauraki Gulf, does not show much inhomogeneity at any one moment owing to the more or less gradual diffusion of fresh water from Auckland Harbour. However there is probably a semi-diurnal cycle of temperature and salinity coincidental with tidal movement in and out of the harbour. Hydrological conditions at Station 2 are fairly consistent with those prevailing in the outer Gulf. Sampling was confined to the upper 20 metres, constituting the photic layer which is markedly turbid down to and including the thermocline. Below the latter is a layer of clear oceanic water with a smaller annual range in temperature. Stations 3 and 4 in the outer approaches to the Gulf are influenced directly by oceanic water, and are thus more closely comparable with Stations 7–10. They are affected in some measure, however, by resident Gulf water (Cassie, in press).

The partially enclosed Wellington Harbour Stations 5 and 6 are the most heterogeneous hydrologically at any one moment owing to turbulent mixing of fresh water from the Hutt River with salt water during tidal circulation (Brodie 1958). Oceanic conditions prevail at stations 7–10. Mean climate at the different sampling sites differs according to latitude and the respective effects of the East Cape Current and the Canterbury Current; but at times all these stations may be influenced by the same body of water and may possess similar plankton populations.

METHODS

Jar samples of surface sea water were used for quantitative counts; the net samples taken below the surface being used for checking species identifications as well as to indicate the abundance and proportion of the commoner phytoplankters in the layers down to 20 metres. The contents of each jar were centrifuged at 1,500 revolutions for 15 minutes and the supernatant liquid siphoned off. Contents left in the centrifuge tubes were then combined in a flat-bottomed, 4-inch specimen tube and allowed to settle. When clear, the liquid was pipetted off to leave the plankters in a volume of just over 1 ml – sufficient to fill a Sedgwick-Rafter counting cell.

Counts were made using a phase contrast × 10 objective. Except where organisms were very dense, the whole cell was examined in strips across the shorter dimension of the cell, by moving the mechanical stage one microscope field at a time. Every species – both plant and animal – was noted, and in the chain-forming diatoms the number of cells in the chain was either counted or estimated according to the species. In dense samples the number of strips counted was reduced.

TABLE 1– Currents, Temperatures, and Salinity Ranges at Stations 1–10

General Locality	Station	Temperature (°C)				Salinity ‰			Currents Influencing Stations 1–10
		Maximum	Minimum	Extreme Range	Monthly Mean Range	Maximum	Minimum	Extreme Range	
Hauraki Gulf	1	22·0	12·0	10·0	.....	35	34	1	Resident Gulf water modified by inflow of fresh water, mostly in winter. Turbidity from suspended organic matter very pronounced (Cassie, In Press).
	2	22·0	12·0	10·0	.....	35·6	34	1·6	Resident Gulf water influenced at times by influx of the East Auckland Current flowing southward (Brodie, In Press). Turbid down to 30 metres. Thermocline present in summer (Cassie, In Press).
	3	20·0	13·0	7·0	5·5 (Garner 1959a)	35·6	35·3	0·3	Oceanic water mainly affected by the East Auckland Current, but at times also by resident Gulf water (Cassie, In Press).
	4	20·0	13·0	7·0	5·5	35·6	35·3	0·3	Similar to 3.
Wellington Harbour	5, 6	19·0	9·5	9·5	7·4 (Skerman 1958)	34·9	29·0	5·9	Resident Harbour water, influenced by large volumes of fresh water, entering mainly from the Hutt River. Tidal circulation complex (Brodie 1958, Cassie 1959). Daily surface temperature varying up to 4·5°C (Skerman 1958). Turbidity moderate to extreme.
S.E. Cook Strait	7, 8	17·0	10·0	7·0	3·6 (Garner 1959a)	35·6	32·7	2·9	Subantarctic water moving northward (the Canterbury Current (Brodie, In Press)) often influences this region. Two centres of upwelling have been reported between here and Kaikoura.
Castle Point	9	Slightly warmer than 7 and 8				.....		.....	Mixed subtropical/subantarctic influence, within zone increased by the Subtropical Convergence (Garner 1959a, b).
Kaikoura	10	Slightly colder than 7 and 8				.....	.....	.....	Directly influenced by the Canterbury Current.

The original volume of the samples was 0·8 litre, but counts of organisms have been adjusted to a litre basis. Figures 2–7 were constructed from graphs based on the original data. Volumes of total plants and animals preserved in the net samples were estimated by reading the number of millilitres against a graduated scale after centrifuging or settling – merely an approximate measure. Actual numbers of cells may be very misleading in portraying dominance; for instance it would take at least a hundred individuals of *Prorocentrum micans* to equal the volume of a single cell of *Biddulphia chinensis*.

HAURAKI GULF

On the east coast of the Auckland Peninsula, the Hauraki Gulf has an area of 1,250 square miles and extends between 36° 12′ and 37° 14′ South Latitude and 174° 40′ and 175° 32′ East Longitude. Its main opening to the ocean is at the north. Geographic and climatic features of this region have been discussed in an earlier account by the author (Dellow 1955). A belt of numerous islands marks a boundary between the outer oceanic, and inner waters of the Gulf.

A summer and a winter population with *Saggita* spp. has been described from the outer Gulf by Fuller (1953). He postulates either an invasion of the Gulf by a single type of oceanic water with salps in summer and sagittas in winter; or else two separate types with salps from one source and sagittas from another.

In the present survey, sufficient numbers of samples were obtained from the inner two of the four Gulf stations to indicate the seasonal trends in diatom and dinoflagellate populations.

Station 1

Station 1 (depth 16 metres) half a mile east of North Head in the Rangitoto Channel, is located over a hard bottom on which the "Tawera - Glycymeris Formation" is said to exist (Powell 1937, 1946). In spite of the hardness of the substrate, samples taken in the water above it were heavily laden with suspended matter possibly from the easily eroded Waitemata Sandstone sediments and from the main Auckland sewer outfall at Orakei. Wallace *et al.* (1958) have shown that sewage is dispersed quite rapidly through the whole depth of Auckland harbour waters, probably as a result of turbulent tidal movement over the uneven bottom. The abundance of detritus frequently made concentrated samples hard to count accurately.

JAR SAMPLES (Fig. 2)

In 1957 the phytoplankton maximum (excluding microflagellates) occurred at the surface during early spring. From August to September *Thalassiothrix nitzschioides, Thalassiosira hyalina* and *Nitzschia seriata* were the dominant diatoms. The latter alone persisted in appreciable numbers until early summer. Only two small dinoflagellates were at all abundant at the surface: *Exuviaella marina* in spring and *Prorocentrum micans* in autumn. Although *Thalassiothrix nitzschioides, Thalassiosira hyalina* and *Biddulphia chinensis* were no denser than 1,000 per litre in autumn and winter they were fairly continuous in their presence throughout these two seasons. *Thalassiosira decipiens* achieved winter dominance in July 1958.

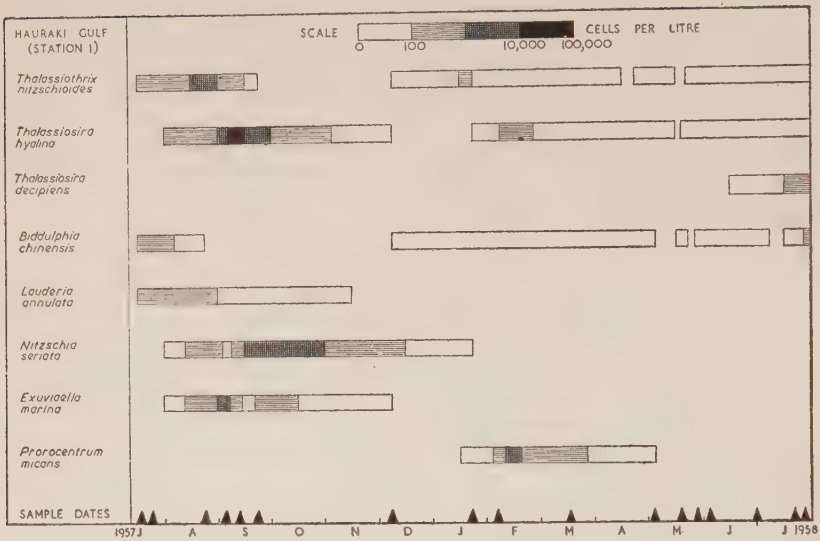


FIG. 2—Hauraki Gulf, Station 1. Number of cells per litre in surface jar samples 1957–58.

NET SAMPLES (Table 2*)

Down to 16 metres *Biddulphia chinensis* was by far the commonest species, *T. nitzschioides* being merely incidental and *Th. hyalina* not pronounced in any sample. *Th. decipiens*, either as single cells or in clusters embedded in a gelatinous mass, achieved dominance just as it did in surface layers during the second winter. *Coscinodiscus oculus iridus* was well represented below the surface, particularly during the first spring. Here too was a distinct period of dinoflagellate abundance from November to January, following the earlier spring diatom peak, the main organisms responsible being the ubiquitous *Ceratium tripos, C. furca* and *C. fusus.*

Throughout the year at this station the majority of species reached their greatest abundance fairly rapidly, and then gradually declined in numbers until their disappearance from the phytoplankton.

Station 2

Maximum depth between Tiri Tiri Island and Whangaparaoa Peninsula is 30 metres and the water is usually clearer than at Station 1. At most times during the year of sampling, this area was extremely rich in variety of diatom species as well as in numbers of individuals.

*Important genera like *Rhizosolenia* and *Chaetoceros* were frequently represented in the net collections. Reference in Tables 2–6 is made only to those organisms most abundant in each sample.

TABLE 2—Station 1, Hauraki Gulf

N50 Net Samples: Dominant Organisms down to 16 metres, 1957–58

5 = very abundant
4 = abundant
3 = common
2 = occasional

SPECIES	JULY		AUG			NOV	JAN		MAR	APR	MAY			JUNE	JULY					
	4	13	11	15	29	20	8	29	3	17	2	10	16	22	9	11	13	18	24	27
Biddulphia chinensis	5	5								5	5				5	5	5	5	5	
Thalassiothrix nitzschioides	4			3	2				3				3							
Ditylum brightwellii	4														3					
Coscinodiscus centralis															3					
Coscinodiscus oculus iridis				4	2		3										3			3
Chaetoceros decipiens				3																
Nitzschia seriata						5														
Thalassiosira decipiens															3		4	5	5	5
Ceratium tripos						4	3													
Ceratium fusus						3	2	2												
Ceratium furca							2													
Copepod nauplii						3														
Copepods										5										
VOLUME (in ml.)	3·1			0·6	0·8	1·0	0·5	0·3	1·0		2·5	5·0		1·0	1·5	2·0	2·5			

JAR SAMPLES (Fig. 3)

In contrast with records from the previous station there was a more pronounced autumn than spring peak in cell numbers of several species. Again the main dominants were *Thalassiothrix nitzschioides, Thalassiosira hyalina* and *Th. decipiens,* all with a more continuous distribution through the year than at Station 1. *Nitzschia seriata* was the only abundant diatom in late

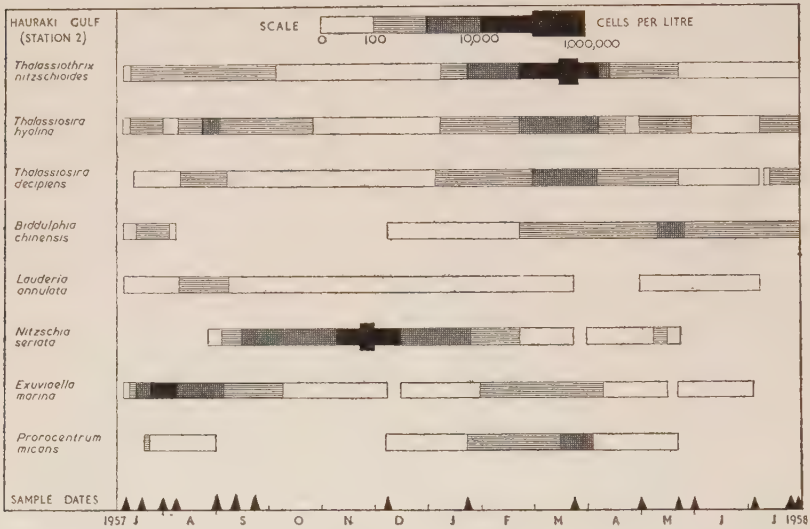


FIG. 3—Hauraki Gulf, Station 2. Number of cells per litre in surface jar samples 1957–58.

spring and early summer, gradually waning as *Biddulphia chinensis* achieved dominance in autumn and persisted in moderately large numbers during the winter months of 1958. *Lauderia annulata* was generally present, though not in any great quantity. The two dinoflagellates *Exuviaella* and *Prorocentrum* flourished in spring and autumn respectively*.

NET SAMPLES (Table 3)

As at Station 1, below the surface *Biddulphia chinensis* and *Thalassiosira decipiens* were the principal species in all save the summer months. *Coscinodiscus oculus iridis* was prominent in early spring, followed by *C. concinnus* from September to November. *C. wailesii* is a winter dominant along with *C. granii.* In November *Nitzschia seriata* achieved profound dominance in subsurface as well as surface layers. *Chaetoceros sociale* appeared in very numerous, contorted chain-masses in May (late autumn). At no time of the year did dinoflagellates predominate here below the surface.

*A bloom occurs when at least one species is present in quantities greater than 10,000 cells per litre. It may last for a relatively short time, as with *Exuviaella* which maintained dominance at this station for a fortnight in July 1957; or it may persist as long as six weeks, like the bloom of *Thalassiothrix nitzschioides* from early February to mid-March 1958.

TABLE 3—Station 2, Hauraki Gulf

N50 Net Samples: Dominant Organisms down to 20 metres, 1957–58

5 = very abundant
4 = abundant
3 = common
2 = occasional

SPECIES	JULY 4	17	AUG 15	29	SEPT 6	NOV 20	MAR 10	APR 10	MAY 2	10	JUNE 22	8	11	JULY 13	18	28
Biddulphia chinensis	5	3						5	5	5	5		5	5	5	5
Biddulphia mobiliensis	3								4							
Coscinodiscus oculus iridis	4	4		5	3							2				
Coscinodiscus excentricus				3												
Coscinodiscus granii	3															
Coscinodiscus concinnus					3	4										
Coscinodiscus wailesii														3		
Thalassiothrix nitzschioides		3		3			4	3								
Nitzschia seriata					4	5										
Thalassiosira decipiens													5	4	4	4
Chaetoceros sociale										5						
Ceratium fusus			2				3									
Ceratium tripos						3						2				
Peridinium pellucidum												2				
Tintinnopsis radix														3		
Copepods																3
VOLUME (in ml.)					0·3	0·6	1·5				4·0	2·0	1·5	2·0	2·0	4·0

Station 3

JAR SAMPLES

In the ten samples examined from Colville Channel, the eastern approach to the Hauraki Gulf (depth 54 metres), several surface diatoms abounded which were not found in any quantity in the inner Gulf. These included *Eucampia zoodiacus, Hemiaulus sinensis, H. hauckii, Melosira sulcata, Leptocylindrus danicus, Rhizosolenia setigera* and *Chaetoceros decipiens.* The highest count was recorded for *Chaetoceros sociale,* with about 11,800 cells per litre on 24 April 1958.

NET SAMPLES

Eucampia was very prolific in July 1957 down to 20 metres, along with *Coscinodiscus oculus iridis* and *Biddulphia mobiliensis.* In August 1957, salps (*Thalia democratica*) were the dominant organisms. *Ceratum fusus* was most abundant in April 1958, which also marked the rise to dominance of *Biddulphia chinensis.* This species maintained its supremacy during the winter.

Station 4

Only four samples were obtained from this station, in Cradock channel between Great and Little Barrier Islands, over a depth of 56 metres.

JAR SAMPLES

Lauderia annulata bloomed at the surface in August 1957, with a maximum count of 37,200 cells per litre.

NET SAMPLES

In the subsurface layers below *Lauderia, Chaetoceros secundum* was the dominant diatom. In December, however, *Coscinodiscus* spp. were abundant, along with *Chaetoceros secundum* and *Biddulphia mobiliensis.* Most unusual was the summer (January) dominance of *B. chinensis* which is normally a winter species in the Gulf. This species also reached a seasonal peak in midwinter, along with *Chaetoceros eibenii, C. secundum, C. decipiens* and *Ditylum brightwellii.*

WELLINGTON HARBOUR (PORT NICHOLSON)

Tidal features of Wellington Harbour have been outlined by Brodie (1958) who has shown that a flooding tide enters the western harbour and passes close to Pt. Halswell with a speed of $\frac{1}{4}$ knot. It continues in a clockwise movement paralleling the inner harbour coastline to follow a southerly direction past Eastbourne. Conditions of flow are reversed during ebb. Especially in winter large volumes of fresh water containing much silt and detritus are transported into the harbour by the Hutt River and Ngauranga Stream. When southerly winds prevail, this water is spread as a thin film over the surface of the harbour water, tending to bank up against the northern end of the harbour. Under northerly conditions on the other hand the Hutt River water traverses a narrow path close to the eastern shoreline.

Stations 5 and 6

Station 5 (depth 22 metres), between Somes Island Lighthouse and Eastbourne Pier, lies roughly in the path of the lower salinity water from the Hutt River. Station 6, midway between Pt. Halswell and Ward Island (24 metres), is traversed directly by the flooding tide. The sea bottom at both stations is mostly soft grey mud (Chart N.Z. 50). With a few exceptions, the same phytoplankton species complex was found at both sites. Both sets of data will therefore be discussed together. Where possible, samples were collected in fair or fine weather, with wind not exceeding Force 4 on the Beaufort Scale. If the sea was at all rough, N50 hauls tended to be oblique.

JAR SAMPLES (Figs 4, 5)

Three noteworthy facts emerge from the available quantitative data:

(1) Greater diatom production in late summer and early autumn than in spring.

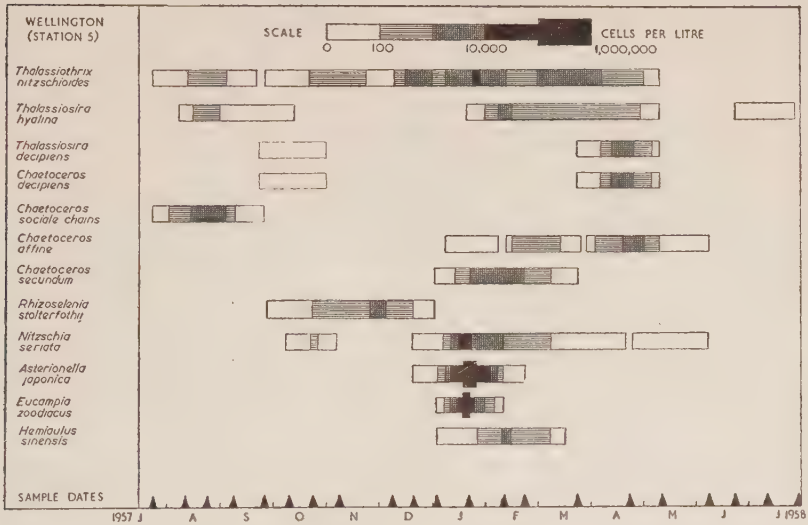


FIG. 4—Wellington Harbour, Station 5. Number of cells per litre in surface jar samples 1957–58.

(2) Absence of any dominant dinoflagellate at the surface.

(3) The abrupt disappearance of nearly every dominant diatom at the surface about the beginning of May 1958, i.e., early midwinter.

(May was by far the windiest month in Wellington during the survey, the hourly average at Kelburn being 14·5 knots. Wind velocity is known to be greater at sea level on the harbour, and could have been a major factor in dispersing surface phytoplankters.)

As in the Hauraki Gulf, the most persistent diatom was *Thalassiothrix nitzschioides*, *Thalassiosira hyalina* being fairly abundant during spring but more so in autumn. The early spring upsurge was marked at both stations by a bloom of *Chaetoceros sociale* so dense that counts of individual cells were

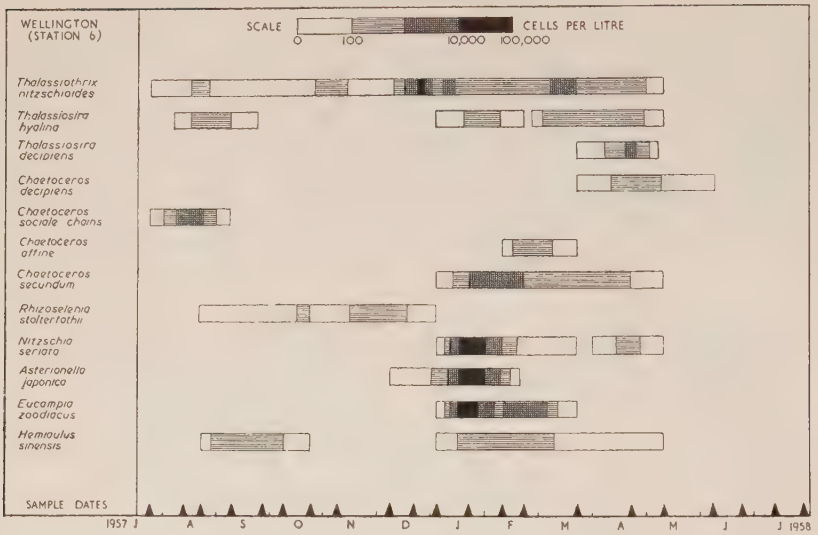


FIG. 5—Wellington Harbour, Station 6. Number of cells per litre in surface jar samples 1957–58.

impossible. This species declined at about the same rate as it rose to dominance. Peak production lasted a fortnight. *Rhizosolenia stolterfothii* and *T. nitzschioides* were the major diatoms in the early summer surface phytoplankton.

A sudden burst of activity occurred in the harbour towards the end of December, when *Asterionella japonica, Nitzschia seriata* and *Eucampia zoodiacus* multiplied within a fortnight to densities as great as 224,000 cells per litre. Actual counts were higher at Station 5 for *Asterionella* and *Eucampia*. Both these species disappeared as quickly as they rose, but other diatom dominants after an initial rapid multiplication tended to linger and fade out more gradually. Duration of a bloom is influenced by local weather conditions, rough seas tending to dissipate existing populations. *Thalassiosira decipiens* and *Chaetoceros affine* rose to prominence during late autumn.

NET SAMPLES (Tables 4, 5)

In subsurface layers, zooplankters – particularly copepods and hydroid medusae (*Obelia* sp. in summer and *Phialidium hemisphaericum* in winter) – formed the bulk of many N50 samples at most times during the year. At Station 6, copepods were very prevalent both during and after the peak abundance of dinoflagellates in September and October, following that caused by diatoms in July and August 1957. In these latter months, *Coscinodiscus wailesii* and *C. centralis* were the dominant phytoplankters down to 20 metres, reappearing again for a brief period in March 1958. *Ceratium furca* was the commonest dinoflagellate in spring, succeeded in later months by *Phalacroma ovum* and *Ceratium tripos*, with *Peridinium ovatum* intruding in January. Notable entrants into the diatom succession in February 1958 were *Streptotheca thamensis* and *Biddulphia mobiliensis*.

TABLE 4—Station 5, Wellington Harbour

N50 Net Samples: Dominant Organisms down to 20 metres, 1957–58

5 = very abundant
4 = abundant
3 = common
2 = occasional

SPECIES	JULY 9	JULY 25	AUG 6	AUG 23	SEPT 10	OCT 22	NOV 19	DEC 5	DEC 19	JAN 6	JAN 22	FEB 6	MAR 5	APR 29	MAY 19	JUNE 5	JUNE 24	JULY 16
Coscinodiscus wailesii ..	3		3	3										3			3	5
Coscinodiscus centralis ..	3		3	3									5					
Coscinodiscus marginatus ..													5				4	3
Coscinodiscus concinnus ..																	4	
Chaetoceros sociale ..			5	4														
Thalassiothrix nitzschioides ..						3				4	3		4					
Asterionella japonica ..										5								
Streptotheca thamensis ..												4						
Biddulphia mobiliensis ..												4						
Biddulphia chinensis ..														4				
Eucampia zoodiacus ..												3		3				
Chaetoceros lorenzianum ..												3						
Chaetoceros secundum ..																	3	
Ceratium furca		3		3										3		3		3
Peridinium ovatum ..											4							
Copepods		5			3	5		5	5						5	3		
Copepod nauplii ..					3	4												
Barnacle nauplii					4	4												
Pleurobrachia sp. ..				5														
Obelia sp.							5											
Tintinnopsis sp.								5										
Echinocardium larvae ..											4							
Phialidium hemisphaericum															5	5	5	5
VOLUME (in ml.) ..			4·4		2·5	2·2	18·9	1·7	6·6	15·0	2·6	3·3	0·6	3·0	2·5	3·5	5·0	7·0

TABLE 5—Station 6, Wellington Harbour

N50: Net Samples: Dominant Organisms down to 20 metres, 1957–58

5 = very abundant 4 = abundant 3 = common 2 = occasional

SPECIES	JULY 9	JULY 25	AUG 6	AUG 23	SEPT 10	SEPT 25	OCT 22	NOV 19	DEC 5	DEC 19	JAN 6	JAN 22	FEB 6	MAR 5	APRIL 8	APRIL 29	MAY 19	JUNE 5	JUNE 24	JULY 16
Coscinodiscus wailesii	5	5	3											5					3	5
Coscinodiscus centralis	5	5	3											5						
Coscinodiscus marginatus																				4
Chaetoceros secundum			5									3							4	
Chaetoceros convolutum			4								3									
Chaetoceros sociale				4	5															
Chaetoceros lorenzianum												3	3							
Chaetoceros affine															4					
Thalassiosira hyalina			3																	
Rhizosolenia imbricata var. *shrubsolei*						3														
Rhizosolenia stolterfothii								2												
Thalassiothrix nitzschioides										3	3	3	3	3						
Thalassiothrix frauenfeldii							3													
Biddulphia mobiliensis													3							
Biddulphia chinensis																4				
Streptotheca thamensis													3			3				
Ditylum brightwellii													3							
Lauderia annulata															3					
Nitzschia closterium						3														
Eucampia zoodiacus																3				
Ceratium furca		4		3	3											3				
Phalacroma ovum						3														
Ceratium tripos								3	3											
Peridinium ovatum												3								
Copepods		5		5	3	5	4	5	3				4				4	3	3	
Copepod nauplii					3	5								3			3			
Barnacle nauplii														3						
Cypris larvae																	3			
Lamellibranch larvae								3						3						
Polychaet larvae																4				
Pleurobrachia sp.				3																
Obelia sp.	3			4		5			5		4	5	3	5			4	5	5	5
Phialidium hemisphaericum																				
VOLUME (in ml.)			2·7	2·8	5·7	10·0	1·5	4·0	2·6	1·3	4·1	1·0	4·0	2·2	10·0		10·0	3·5	10·0	13·5

S.E. Cook Strait

Owing to seasonal changes in trawling grounds by the *Thomas Currell* no one set of samples from Stations 7–10 covers a whole year. Depths below these sampling sites are greater than those in the Hauraki Gulf and Wellington Harbour, and their longer distance from land places them in a more oceanic hydrological environment. This is particularly applicable to Stations 7 and 8.

Station 7

Phytoplankton samples were obtained south of Cape Campbell and southeast of Needles Point over depths varying from 60 to 130 metres. A single haul was made here in July 1957, but reasonably continuous records were obtained between December 1957 and June 1958.

Jar Samples (Fig. 6)

In general, cell numbers were much smaller than those in samples from the Hauraki Gulf and Wellington Harbour. *T. nitzschioides* again persisted longer than any other diatoms, but was never more dense than 200 cells per litre. *Corethron criophilum* and *Skeletonema costatum* attained dominance in summer (December/January) giving way as autumn advanced to *Melosira granulata* var. *angustissima* – usually a denizen of fresh-water (Nygaard 1945).

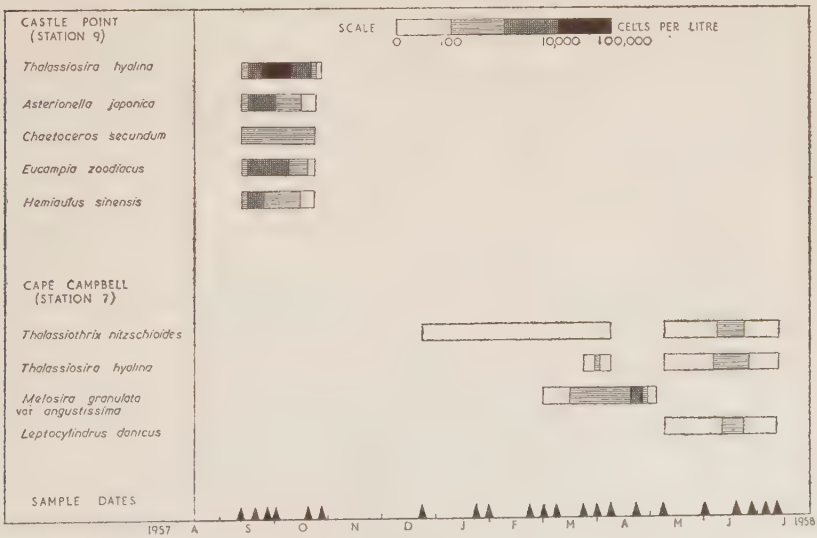


Fig. 6—Castle Point, Station 9; Cape Campbell, Station 7. Number of cells per litre in surface jar samples 1957–58. (Gaps for Station 9 between November and July, and for Station 7 between August and December indicate that no samples were taken in that area.)

This species appeared frequently during early autumn in the jar samples. It is known to have produced several million cells per litre in the Wainuiomata Reservoir and on several occasions has appeared in Wellington Harbour samples. Quite possibly it was also of fresh-water origin in the Cape Campbell region. *Leptocylindrus danicus* rose to prominence later in the succession. A further fresh-water representative common in autumn samples was *Dinobyron divergens.*

Thalassiosira hyalina appeared more continuously in winter 1958, but no more than 500 cells per litre were recorded.

NET SAMPLES (Table 6)

Net hauls revealed a striking dominance of copepods, nauplii, and other zooplankters such as mysids, gastropod larvae, and *Pyrosoma atlanticum* in summer and autumn. It should be mentioned in this respect that nearly all the 1958 net samples at this station were taken at night (between 6 p.m. and 3 a.m.). In mid-December 1957 a scattered dinoflagellate population was represented by *Dinophysis fortii, Peridinium pedunculatum* and *Ceratium arietinum.* These gave way rapidly to copepods which were by far the most important plankters until May 1958, when there was a resurgence of diatoms, heralded by *Corethron, Ditylum, Stephanopyxis orbicularis* and *Coscinodiscus concinnus.* These were succeeded by *Rhizosolenia styliformis, Leptocylindrus* and later *Chaetoceros concavicorne.*

Station 8

Almost 13 miles due east of Cape Campbell, this station is located near the centre of the southern approaches to Cook Strait, over a depth of 120–130 metres.

JAR SAMPLES

Mid-August 1957 saw a dominance of *Thalassiosira hyalina* and *Th. rotula,* the latter as dense as 1,400 cells per litre. *Th. condensata* achieved a peak of over 2,000 cells per litre in January 1958, with *Lauderia annulata* and *Thalassiosira decipiens* almost as abundant. *Leptocylindrus* and *Melosira granulata* var. *angustissima* were common in March. There were no dominant surface dinoflagellates in any of the 10 samples from this station. Jar samples were poor in comparison with those obtained during 1944–45 in Cook Strait by Crawford (1947), whose stations, however, were within a few miles of Wellington Harbour entrance (pers. comm.). She noted an August-September diatom increase to counts of up to 10,930 cells per litre, together with noticeable quantities of *Dinophysis, Peridinium,* and *Ceratium* spp. Crawford records a late summer maximum of small-celled species, including *Skeletonema, Nitzschia seriata, N. closterium* and *Leptocylindrus* (31.1.45: 22,840 cells per litre).

TABLE 6—Station 7, Cape Campbell

N50 Net Samples: Dominant Organisms down to 20 metres, 1957–58

5 = very abundant
4 = abundant
3 = common
2 = occasional

SPECIES	JULY	DEC		JAN			FEB			MARCH				APR		MAY	JUNE			
	16	5	12	8	16	23	13	20	27	5	13	20	27	9	22	16	4	10	17	25
Thalassiothrix nitzschioides	2															2	2	2		
Chaetoceros convolutum	2																			
Chaetoceros concavicorne																				2
Chaetoceros secundum																		2		
Corethron criophilum		3														2			2	
Skeletonema costatum		2																		
Licmophora lynghyei												2								
Rhizosolenia stolterfothii																2				
Rhizosolenia styliformis																	3			
Ditylum brightwellii																2				
Coscinodiscus concinnus																2	3			
Stephanopyxis orbicularis																2				
Leptocylindrus danicus																	4			
Thalassiosira decipiens																				2
Dinophysis fortii			2																	
Peridinium pendunculatum			2																	
Ceratium arietinum			2																	
Goniaulax monacantha												2								
Tintinnopsis radix									3											
Dictyocha fibula												2								
Globigerina bulloides																2				
Copepods and nauplii			5	5	5	5	5	5	5	5	5	5	5					3	3	4
Salps				3	3	3														
Mysids			5			3														
Pyrosoma atlanticum						5														
lamellibranch larvae															5					
VOLUME (in ml.)		0·6		4·5	5·6		0·6	1·5		3·6	10·0					1·5	2·0	4·0	0·5	

NET SAMPLES

Diatoms not well represented in the August surface samples but common in subsurface layers including *Ditylum, Biddulphia chinensis, T. nitzschioides* and *Eucampia zoodiacus.* From September to November animals dominated the plankton – chiefly copepods (some heavily epiphytised by a stalked, naviculoid diatom), nauplii and salps (*Thalia democratica*). Together with mysids and hydroid medusae these were again prevalent in March.

Station 9

This station was located east of Castle Point over depths varying from 56 to 120 metres.

JAR SAMPLES (Fig. 7)

From 4 of the 6 samples collected between July and October 1957, there was evidence of a pronounced spring bloom. Within a week in September total cell counts per litre rose from 60 to 60,000, increasing to 76,000 in the following week and dropping to less than 200 during the next fortnight. By 10 October total cell numbers were as low as 30. A variety of diatoms contributed to the bloom, the most conspicuous being *Thalassiosira hyalina, Th. condensata, Asterionella japonica, Hemiaulus sinensis, Eucampia zoodiacus* and *Chaetoceros secundum.*

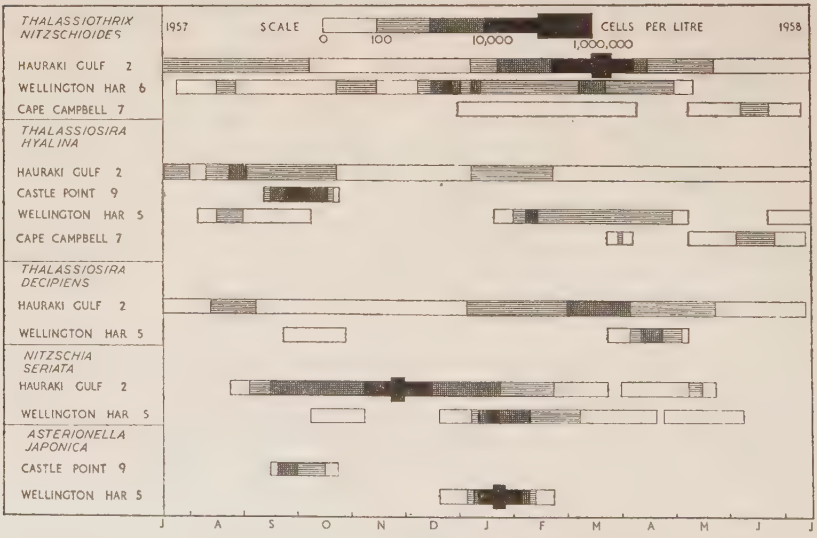


FIG. 7—Duration and abundance of four dominant surface diatoms in the Hauraki Gulf, Wellington Harbour, and Cook Strait.

NET SAMPLES

At the zenith of the spring bloom, diatoms most abundant below the surface were *Th. hyalina, Stephanopyxis orbicularis, Asterionella, Eucampia, Lauderia,* and *Rhizosolenia imbricata* var. *shrubsolei.* Dinoflagellates assumed

dominance almost immediately after the decline of diatoms. Early in October there appeared first *Peridinium depressum* and *P. ovatum* and then a week later *Ceratium tripos, C. porrectum, C. furca,* and *C. arietinum.* A sample taken in July 1958 indicated a *Coscinodiscus* population, mainly *C. wailesii* and *C. concinnus.*

Station 10

The southernmost station in this survey, about 10 miles north-east of Kaikoura Peninsula and 5–6 miles offshore, was sampled over a depth varying from 60 to 80 metres.

Jar Samples

As at Stations 7 and 8 total numbers were low, the largest count being 750 cells per litre for *Melosira granulata* var. *angustissima* in March 1958. *T. hyalina* and *Skeletonema* were conspicuous at the surface in summer (December). A further freshwater diatom, *Asterionella gracillima,* appeared in March, along with the tiny filaments of *Melosira.* Towards the end of May, *Coscinodiscus* spp. (*C. oculus iridis, C. centralis. C. radiatus*) were the only diatoms in any quantity.

Net Samples

Copepods and their nauplii predominated in all net samples save the December one, which consisted solely of diatoms – mostly *Chaetoceros affine* with a moderate amount of *C. decipiens, T. nitzschioides, Corethron* and *Skeletonema.* The tintinnid *Codonella robusta* was a prominent member of the subsurface phytoplankton in April 1958.

Discussion

Figure 7 shows the duration and abundance of dominant surface diatoms common to two or more localities. It is evident that in the Hauraki Gulf, certain diatoms can maintain an appreciable population for a longer period of time than in either Wellington Harbour or the more open waters in S.E. Cook Strait.

Thalassiothrix nitzshioides emerges as the most widespread and constant member of the surface phytoplankters, while *Thalassiosira hyalina* comes to a close second. Both these species were more prolific in the Gulf and Harbour areas than in Cook Strait. (Although not recorded at Station 7, *Th. hyalina* and *Th. rotula* achieved a spring maximum of 1,750 cells per litre at Station 8 on 28 August 1957.) From the data available it is difficult to generalise about the effect of latitude on time of blooming of a particular species. *T. nitzschioides* for instance was most abundant in Wellington Harbour during midsummer, but not until the autumn diatom maximum in the Hauraki Gulf. *Nitzschia seriata* on the other hand reached its peak in the Gulf in November and during January in Wellington Harbour. *Thalassiosira condensata* was most dense off Castle Point in September, but not till January in Cook Strait. It was not found as a dominant in the Gulf phytoplankton. Earlier dominance of this species, together with *Nitzschia seriata* and *Asterio-*

nella japonica at Castle Point than in Wellington Harbour could be evidence for a summer penetration into Cook Strait and later into the harbour of a tongue of water from the warm East Cape Current (cf. Garner 1959a).

Chaetoceros sociale produced a spectacular bloom in Wellington Harbour early in August 1957. The same species was conspicuous in two Gulf samples collected during autumn of 1958. Brewin (1952) regards *C. sociale* as a herald of spring in Otago Harbour, where it predominated in July 1946.

To summarise the situation at the surface, diatoms were the chief phytoplankters in all three localities, dinoflagellates being either secondary in importance or absent. Only Station 2 in the Hauraki Gulf provided the small dinoflagellates *Prorocentrum micans* and *Exuviaella marina* in any quantity. Both spring and autumn maxima occurred in the Hauraki Gulf, spring producing a greater number of cells in the Rangitoto Channel, in contrast with the autumn maximum in Tiri Passage. Living diatom cells were present in appreciable quantities in the Gulf throughout the winter. Five degrees south in Wellington Harbour, most intense diatom activity took place in late summer/early autumn. Dominant species lingered after a rapid rise in cell numbers, but most of these disappeared abruptly about the beginning of May 1958. Winter gales are thought to be largely responsible for this. About the southern approaches to Cook Strait numbers of cells per litre were much smaller (seldom over 1,000). A spring abundance of *Thalassiosira* spp. was demonstrated at Station 8 and a late autumn increase in cell division of several species, particularly those with fresh-water affinities (*Melosira granulata* var. *angustissima, Dinobryon divergens*).

Below the surface the pattern of dominance assumed a different facies. In the Hauraki Gulf *Biddulphia chinensis* was of prime importance in the winter months, although a midsummer sample from Station 3 displayed great quantities of it. In Wellington Harbour however, *B. chinensis* was less conspicuous, only once appearing densely: in April 1958.

Coscinodiscus was a much more prevalent genus in subsurface phytosynthetic layers, especially in winter months and in land-protected localities. The principal species in the Gulf and Wellington were *C. marginatus, C. centralis, C. wailesii, C. oculus iridis,* and *C. concinnus. Thalassiosira decipiens. Coscinodiscus.* and *B. chinensis* formed the bulk of the subsurface winter phytoplankters in the Gulf, whereas in Wellington Harbour *Chaetoceros* spp. (*C. secundum, C. sociale, C. convolutum*) co-dominated with *Coscinodiscus.* Two spring species prominent in S.E. Cook Strait but not in Wellington Harbour were *Rhizosolenia styliformis* and a long, narrow form of *Corethron criophilum.* This area was distinguished by the vast numbers of copepods and other zooplankters from December to March, and at most other times of year appeared to be greatly in excess of the diatom and dinoflagellate populations. Peak animal production in Wellington occurred sooner (September to December). By contrast, the Hauraki Gulf provided but one net sample in which copepods exceeded the phytoplankters.

More distinctive of each area were the dinoflagellates, apart from three ubiquitous species. Those with a restricted distribution included *Peridinium pellucidum* in the Gulf, *Phalacroma ovum* in Wellington and *Dinophysis fortii, Peridinium pedunculatum* and *Ceratium arietinum* in Cook Strait. The latter was also recorded from Castle Point.

The cosmopolitan *Ceratium tripos, C. furca.* and *C. fusus* abounded in the Gulf from November to January. *C. furca* was an early spring member in the Wellington subsurface phytoplankton but *C. tripos* flourished about the same time as in the Gulf.

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APPENDIX

Species Recorded from Net and Jar Samples at 10 New Zealand Stations between July 1957 and July 1958

L = locality
A = abundance (maximum for month)
N = number of samples

Locality	Stations	Symbol
Hauraki Gulf	1–4	H
Wellington Harbour	5–6	W
Cook Strait	7–8	C
Castle Point	9	P
Kaikoura	10	K

Abundance Scale	Net	Jar
5	very abundant	>1,000
4	abundant	101–1,000
3	common	51–100
2	occasional	11–50
1	scarce	2–10
r	one specimen only	1

SPECIES	JULY			AUG			SEPT			OCT			NOV			DEC			JAN			FEB			MAR			APR			MAY			JUNE			JULY		
	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N
BACILLARIOPHYCEAE																																							
Achnanthes brevipes Agardh	H	r	6							P	1	2																											
Achnanthes longipes Agardh	H	r	6	H	1	11	C	1	1	W	3	4	H	2	2	W	3	4	H	1	4	W	4	2	C	r	3	H	1	3				H	1	4	H	1	12
	W	1	2	W	3	4													W	2	4																	
Actinoptychus senarius (Ehr.) Ehr. ..	H	r	6				C	1	1																H	1	1	H	1	3	H	r	6	H	1	4	H	r	12
																																		W	1	4	P	1	1
Amphiprora alata (Ehr.) Kütz.				H	r	11																																
Amphora chinensis A. Schmidt	H	2	6	H	2	11							H	3	2	H	3	1	H	3	4				H	4	1	H	1	3	H	1	6	H	1	4	H	2	12
Amphora crassa Hanna & Grant																																		H	r	4			

SPECIES	JULY			AUG			SEPT			OCT			NOV			DEC			JAN			FEB			MAR			APR			MAY			JUNE			JULY		
	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N
Asterionella japonica Cleve	W	1	4	C	1	3	P	5	2	W	1	4	C	1	1	W	4	4	W	5	4	C	2	3	H	1	1	H	4	3	H	4	6	H	3	4			
																C	1	2	C	2	2				K	2	2												
																K	1	1																					
Asterionella gracillima (Hantz.) Heiberg ..							W	r	4																C	1	6	H	2	3				H	2	4	H	1	12
																									K	3	2	W	1	2				C	2	4			
																												C	2	3									
																												K	1	2									
Asterionella karyana Grun.																															C	r	1						
Asteromphalus flabellatus Grev.				H	2	11							H	r	2				H	1	4				H	4	1	H	1	3				H	1	4	H	2	12
Asteromphalus hookeri Ehr.																																		C	r	4			
Bacillaria paxillifer (Müll.) Nitzsch ..	H	r	6																W	1	4													H	r	4	H	1	12
Biddulphia aurita (Lyng.) B. & G. *var. obtusa* (Kütz.) Hust.																W	4	4				W	5	2				W	4	4				W	3	4	H	1	12
																																		C	1	4			
Biddulphia chinensis Grev.	H	5	6	H	1	11	W	r	4	P	1	2	C	r	1	H	5	1	H	1	4	W	1	2	H	4	1	H	5	3	H	5	6	H	5	4	H	5	12
	W	2	4	W	2	4	P	2	2																W	1	2	W	4	4	W	3	2	W	1	4			
	C	2	2	C	3	3																						K	1	2									
Biddulphia mobiliensis (Bail.) Grun. ..	H	3	6	H	1	11				P	r	2	H	1	2	H	1	1	H	1	4	W	4	2	H	3	1	H	3	3	H	4	6	H	2	4	H	1	12
	C	1	2													W	r	4	W	1	4				W	2	2	W	2	3	W	2	2	W	2	4	W	r	2
																C	1	2							C	1	6	K	1	2				C	r	4	P	1	1
																K	2	1																					
Cerataulina pelagica (Cleve) Hendey ..				H	1	11	H	r	1	P	1	2				W	1	4	H	1	4	W	2	2				H	2	3	H	4	6	W	3	4	H	2	12
				W	2	4													W	4	4							W	4	4	W	2	2				W	1	2
Chaetoceros affine Lauder				W	2	4	W	3	4							C	1	2	W	2	4	W	4	2	W	1	2	W	5	4	C	1	1	W	2	4	H	1	12
																K	5	1							C	1	6	K	r	2							W	1	2
																									K	3	2	C	1	3									
Chaetoceros atlanticum Cleve																															H	r	6						
Chaetocerus compressum Lauder																H	1	1							H	4	1							H	4	4	W	2	2

| | | |

SPECIES	JULY			AUG			SEPT			OCT			NOV			DEC			JAN			FEB			MAR			APR			MAY			JUNE			JULY		
	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N
Corethron criophilum Castr.				H	r	11	W	r	4	P	1	2				W	1	4	W	2	4	W	2	2	W	1	2	W	2	4	W	2	2	H	1	4	W	1	2
				C	1	3										C	4	2	C	1	2				C	r	6				C	2	1	W	4	4	P	r	1
																K	4	1																				
Coscinodiscus asteromphalus Ehr.							H	1	1	W	2	4	H	1	2										C	1	6							H	1	4	H	1	12
Coscinodiscus centralis Ehr.	W	3	4	H	2	11	H	r	1							W	2	4	H	1	4	W	1	2	H	2	1	H	1	3	H	1	6	H	1	4	H	1	12
				W	3	4	W	2	4																W	5	2	W	4	4	W	2	2	W	2	4	W	1	2
																									C	2	6	C	1	3	C	2	1	C	r	4			
																												K	2	2	K	2	2						
Coscinodiscus concinnus W. Smith	H	3	6	H	3	11	W	1	4							W	1	4	H	1	4							W	1	4	C	2	1	H	2	4	H	2	12
	W	2	4	W	2	4	P	1	2							K	1	1															W	4	4	W	2	2
																																	C	1	4	P	5	1
Coscinodiscus excentricus Ehr.	H	1	6	H	3	11							H	2	2	W	1	4	H	2	4				W	1	2				H	1	6	C	1	4	H	1	12
																			W	1	4				K	1	2				C	4	1						
Coscinodiscus granii Gough				H	1	11										H	1	1	H	2	4	W	1	2	H	3	1	H	2	3				H	2	4	H	1	12
																									W	2	2												
Coscinodiscus marginatus Ehr.	H	1	6	H	3	11				W	1	4	H	1	2	W	1	4							W	1	2	W	1	4	W	1	2	W	4	2	W	5	2
				W	2	4							W	2	2																					P	1	1
Coscinodiscus oculis iridis Ehr.	H	4	6	H	5	11	H	3	1	W	2	4	H	4	2	H	2	1	H	3	4	W	1	2	H	2	1	H	2	3	H	1	6	H	2	4	H	3	12
							W	1	4				W	2	2	W	2	4	W	1	4				W	1	2	W	2	4	W	2	2	W	1	4			
																C	r	2										C	1	3	C	1	1	C	r	4			
																												K	1	2	K	2	2						
Coscinodiscus radiatus Ehr.	H	1	6	H	1	11	H	1	1	W	1	4	H	1	2	W	1	4	H	1	4				H	4	1	H	2	3	H	1	6	H	2	4	H	1	2
							W	2	4				W	2	2										W	2	2	W	3	4	C	1	1	W	1	4	W	1	2
							P	r	2																			C	1	3	K	2	2	C	1	4	C	1	1
Coscinodiscus strigillatus Witt.																W	1	4																				
Coscinodiscus subtilis Ehr.				C	1	3	P	1	2																														
Coscinodiscus wailesii Gran & Angst	H	r	6	H	1	11	H	3	1				H	4	2	H	2	1	H	1	4				H	1	1	H	1	3	H	1	6	H	2	4	H	3	12
	W	2	4																						W	1	2	W	3	4				W	5	4	W	5	2
	C	2	2																																	P	3	1

| | | |

SPECIES	JULY			AUG			SEPT			OCT			NOV			DEC			JAN			FEB			MAR			APR			MAY			JUNE			JULY		
	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N
Melosira moniliformis (Müll.) Agardh ..	P	1	1																						K	1	2												
Melosira sulcata (Ehr.) Kütz.	H	2	6	H	2	11	H	1	1				C	1	1	H	2	1	H	1	4				H	4	1	H	3	3				H	1	4	H	2	12
	C	1	2	W	2	4	W	1	4							C	2	2	W	2	4							W	4	4				C	3	4			
																												K	1	2									
Navicula membranacea Cleve							W	1	1				H	1	2	H	2	1	H	2	4				C	1	6	W	2	4	H	2	6	H	2	4	H	r	12
																									K	2	2				C	1	1						
Navicula lyra Ehr.	H	r	6																																		H	1	12
Navicula cyprinus (W. Smith) Boyer ..																												W	1	4									
Nitzschia closterium (Ehr.) W. Smith ..	H	1	6	W	1	4	W	3	4	W	r	4	H	4	2	H	1	1	H	2	4	W	5	2	W	1	2	H	1	3	H	1	6	H	2	4			
	W	1	4	C	1	3	C	1	1	P	2	2	W	r	2	W	3	4	W	5	4	C	1	3	C	1	6	W	1	4	C	1	1	C	2	4			
							P	4	2				C	3	1	C	1	2							K	1	2	C	1	3									
																K	2	1										K	1	2									
Nitzschia seriata Cleve	H	1	6	H	4	11	H	4	1	W	3	4	H	5	2	H	3	1	H	2	4	W	4	2	C	2	6	W	2	4	H	4	6				H	2	12
							W	2	4				W	1	2	W	2	4	W	5	4							K	1	2									
							P	4	2																														
Plagiogramma sp.?	H	4	6	H	2	11							H	4	2	H	1	1	H	2	4				W	1	2	H	4	3	H	1	6	H	4	4	H	3	12
Planktoniella sol (Wall.) Schütt							P	1	2																											H	r	12
Pleurosigma affine Grun.	H	3	6	H	2	11	W	1	4	W	1	4	H	2	2	W	2	4	H	1	4	W	2	2	H	3	1	H	1	3	H	2	6	H	1	4	W	1	2
				W	1	4	P	1	2										W	3	4				W	1	2	W	1	4	C	1	1	C	1	4			
Pleurosigma angulatum (Queckett) W. Smith ..													H	1	2				H	2	4	C	1	3	H	2	1	H	1	3	H	1	6	H	2	4	H	1	12
																									W	2	2							W	1	4	W	1	2
																									C	1	6										P	1	1
																									K	1	2												
Pleurosigma balticum (Ehr.) W. Smith ..				H	1	11	H	r	1							H	1	1							H	2	1	H	1	3	H	2	6	H	2	4	H	1	12
							P	1	2							W	r	4							W	1	2												
Pleurosigma formosum W. Smith	H	1	6	H	2	11	H	1	1				H	r	2	H	1	1	H	2	4				H	1	1	H	2	3	H	2	6	H	1	4	H	1	12
Pleurosigma naviculaceum Bréb.	H	2	6	H	2	11	P	1	2	W	1	4	H	2	2	H	2	1	H	2	4	W	1	2	H	4	1	H	1	3	H	3	6	H	4	4	H	2	12
													W	1	2	W	1	4							W	2	2	W	2	4	W	r	2				W	1	2
																																					P	1	1

| | | |

SPECIES	JULY			AUG			SEPT			OCT			NOV			DEC			JAN			FEB			MAR			APR			MAY			JUNE			JULY		
	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N
Thalassiosira decipiens (Grun.) Jorg. ..	H	3	6	H	2	11	W	1	4	W	2	4	H	2	2	H	1	1	C	4	2				H	5	1	H	4	3	H	4	6	H	4	4	H	5	12
							P	4	2																			W	5	4				W	1	2	W	r	2
																																		C	2	1			
Thalassiosira hyalina (Grun.) Gran ..	H	4	6	H	5	11	W	2	4	W	1	4	H	2	2	H	2	1	H	4	4	W	3	2	H	3	1	H	4	3	H	5	6	H	3	4	H	5	12
	C	1	2	W	4	4	P	5	2	P	2	2	C	1	1	W	1	4	W	4	4	C	1	3	W	4	2	W	4	4	C	3	1	W	2	1	W	2	2
																C	4	2	C	1	2				C	1	6				K	2	2	C	4	4	C	1	1
																K	4	1							K	4	2										P	4	1
Thalassiosira rotula Meun.				C	4	3	P	5	2	P	2	2							H	1	4	W	1	2				H	2	3	H	4	6				P	1	1
																												K	r	2	C	3	1						
Thalassiothrix frauenfeldii Grun.							W	1	4	W	3	4	K	1	1	H	2	1	W	2	4	W	4	2	C	2	6	H	2	3	H	1	6	H	1	4	H	2	12
																W	2	4	C	1	2	C	1	3	K	1	2	W	1	4									
																C	1	2										C	1	3									
																												K	1	2									
Thalassiothrix nitzschioides Grun.	H	4	6	H	5	11	H	2	1	W	4	4	H	2	1	H	4	1	H	3	4	W	4	2	H	5	1	H	4	3	H	4	6	H	2	4	H	2	12
	W	2	4	W	4	4	W	2	4	P	3	2	W	2	2	W	5	4	W	5	4	C	2	3	W	5	2	W	4	4	W	1	2	C	4	4	P	1	1
	C	3	2	C	3	3	C	4	1	K	1	1				C	2	2	C	2	2				C	2	6	C	1	3	C	2	1						
							P	4	2							K	4	1							K	3	2	K	1	2	K	1	2						
Triceratium alternans Bail.				C	1	3				W	1	4				W	1	4	W	2	4				W	3	2	H	1	3				H	r	4	W	1	2
																												W	2	4				W	2	4			
																												K	1	2									
Triceratium favus Ehr.																									C	1	6												
Tropidoneis lepidoptera (Greg.) Cleve ..																												H	1	3									
																												W	3	4									
DINOPHYCEAE																																							
Ceratium arietinum Cleve										P	1	2				C	2	2																					
Ceratium buceros f. molle (Kof.) Schill. ..							C	1	1							H	1	1																					
Ceratium buceros f. tenue (Ost. & Sch.) Schill...	H	1	6																																				
Ceratium furca (Ehr.) Clap. & Lach. ..	W	4	4	H	1	11	W	2	4	W	2	4	W	2	2	H	1	1	H	3	4	W	1	2	H	4	1	H	2	3	H	r	6	H	1	4	H	1	12
	C	1	2	W	3	4	C	1	1	P	1	2				W	1	4				C	1	3	C	r	6	W	3	4	W	1	2	W	1	4	W	3	2
	P	1	1				P	1	2							K	1	1										K	1	2				C	r	4	P	1	1

| | | |

SPECIES	JULY			AUG			SEPT			OCT			NOV			DEC			JAN			FEB			MAR			APR			MAY			JUNE			JULY		
	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N
Peridinium claudicans Paulsen				H	1	1	H	r	1																				H	1	6				H	1	12
Peridinium conicum (Gran) Ost & Schm. ..																												W	1	4									
Peridinium depressum Bailey				W	1	4	C	2	1	P	4	2	W	1	2	C	1	2											H	r	6	H	1	4	H	1	12
							P	1	2																						W	1	4	W	r	2
																															C	1	4						
Peridinium granii Ost.																												W	1	4				W	1	4			
Peridinium leonis Pav.				H	1	11																						H	1	3				H	1	4	H	2	12
																												W	1	4									
Peridinium marielebourae Paulsen																									H	1	1	H	1	3				H	1	4			
Peridinium ovatum (Pouchet) Schütt ..				H	3	11	W	1	4	P	3	2	H	2	2				W	4	4	W	1	2	W	1	2	H	1	3	W	r	2	H	r	4	H	r	12
				W	1	4	C	2	1										C	1	2													W	1	4	P	1	1
				C	1	3	P	2	2																														
Peridinium pallidum Ost.																																		C	r	4			
Peridinium pedunculatum Schütt				H	1	11										C	2	2	H	3	4				C	1	3											
																			W	2	4																	
Peridinium pellucidum (Bergh) Schütt ..				H	1	11							H	2	2																			C	1	4	H	2	12
Peridinium pentagonum Gran				H	2	11										H	1	1	H	3	4	W	2	4	H	2	1	H	1	3								
																			W	2	4																	
Peridinium steinii Jorg.	H	2	6	H	1	11																									H	r	6						
Phalacroma ovum Schütt				W	1	4	W	1	4																C	r	6											
Prorocentrum micans Ehr.	H	3	6	H	2	11	W	1	4	W	1	4				H	1	1	H	5	4	W	2	2	H	1	1	H	5	3	H	1	6	H	1	4	H	1	12
	W	1	4	W	1	4													W	1	4				C	r	6											
Prorocentrum rostratum Stein													H	1	2	W	2	4	W	1	4	W	1	2	C	1	6	C	2	3								

| | | |

SPECIES	JULY	AUG	SEPT	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUNE	JULY
	L A N	L A N	L A N	L A N	L A N	L A N	L A N	L A N	L A N	L A N	L A N	L A N	L A N
CRUSTACEA													
Penilia avirostris Dana	H r 6									H r 3			
ECHINODERMATA													
Echinocardium cordatum (Pennant) – larvae ..							W 3 4	W 2 2					
TUNICATA													
Thalia democratica (Forskal)	H 2 6	H 2 11			C 5 1		C 4 2						
Pyrosoma atlanticum Peron											C 5 1		
APPENDICULARIA													
Oikopleura sp.		H 1 11							H 1 1	H r 3 K 1 2	C 1 1	H 1 4	H 1 12 W r 2
CHAETOGNATHA													
Sagitta sp.	H r 6 C r 2									K 1 2			
CTENOPHORA													
Pleurobrachia sp.		H 1 11 W 5 4			C r 1					K r 2	W 2 2	W 2 4 C r 4	

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